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14. ABSTRACT The project investigated the development of ligand linked Flutamide analogs for complexing a M(CO)3 (Re,99mTc) organometallic species to target prostate cancer for imaging and therapy. The project has been successful in developing and testing new synthetic strategies for this application. General methods were established for preparing the complexes in excellent yields(>95%) at (10-4,10-5 M) ligand concentration, testing the stability of the complexes (pH, temperature) and in vitro (serum, AR +/- prostate cancer cells). First generation 99mTc flutamide analogs were examined. The results indicated the complexes formed with tridentate ligands (i.e., cysteine, histidine) were successfully prepared and maintained stability. The "2+1" approach analogs were prepared, however, failed to maintain the complex conformation, when examined under biological conditions. Second generation and addition coordination modes are also being investigated.

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Imaging and therapy of Prostate cancer

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Introduction

The focus of the research as highlighted in the proposal is the development of new diagnostic agents for identifying and probing prostate cancer. Flutamide, a non steroidal antagonist of the androgen receptor, is a current medical treatment of prostate cancer.[1] The proposed work involves the synthesis of novel modified flutamide derivatives that incorporates radionuclides (^{99m}Tc, ^{188}Re) into the framework of the system as unique organometallic species, $M(CO)_3^+$. These radionuclides have an important contribution to the molecule by providing a mechanism to directly image and therapeutically treat prostate cancer at the primary and potentially secondary sites. The potential outcome of this work would be the development of radioactive incorporated flutamide compounds that can be used to actively monitor existing treatment protocols and to provide an enhanced therapeutic value in conjunction with associated emissions. The compounds may also have potential use is evaluating hormone refractory syndrome as cancer cells become drug resistant or mutations occur.

Body

The work conducted during year three has continued to meet the objectives of the proposal. The work during year three has combined several of the objectives of year two and three. The primary focus of year three has correlated with the objectives listed in the scope of work initiate the testing of compounds, continued synthesis of flutamide modified compounds and characterization Re and Tc-99m of complexes. One of the major objectives achieved during this time period was the evaluation and redirection of the project based on the observed results. The experiments and methods utilized within the time period are discussed in conjunction with the proposal objectives illustrate in bold.

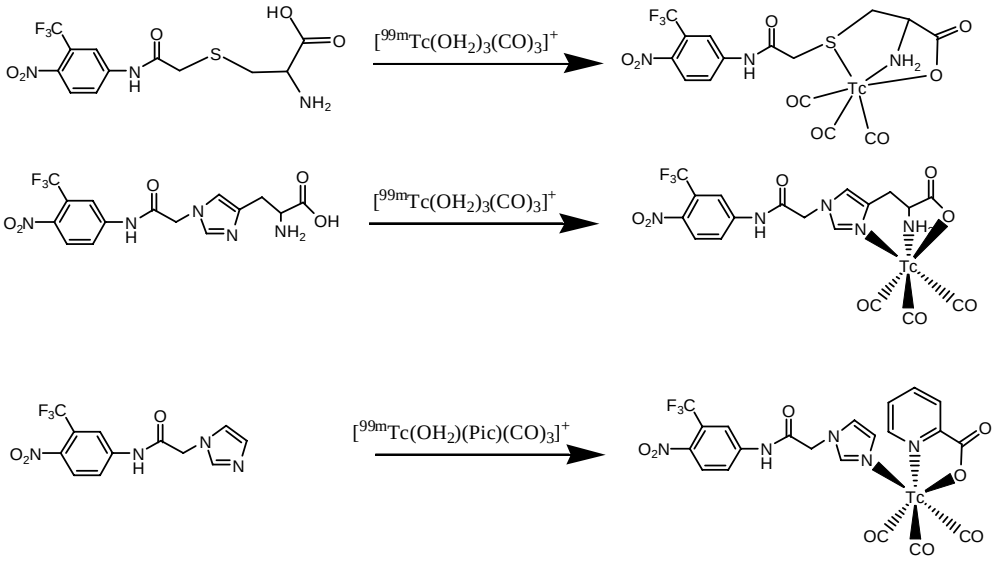
Year Three Objectives

- **Compare and contrast the various methods and complexes tested (Year 3)**

Work conducted in year three focused on the completion of the ligand linked flutamide species and the corresponding metal complexes. The first generation of preliminary ligands (histidine, cysteine, imidazole) linked with flutamide were prepared utilizing conditions developed in year 2 and were fully characterized.[1-3] The corresponding metal complexes to these first generation compounds were also investigated. The complete discussion of the compounds can be found in the article accepted by Bioconjugate Chem (attached). Our initial investigation focused on preparing the Re and Tc-Flutamide compounds to evaluate tridentate and “2+1” ligand approach. Our

hypothesis suggested that both the tridentate and “2+1” ligand approaches would be acceptable for preparing Re and Tc-Flutamide compounds. From a chemical perspective, tridentate and “2+1” ligand approaches toward complexing $M(CO)_3$ had similar properties. However, further investigation of the compounds revealed that the two approaches had significantly different stabilities.

Figure 1. First generation ^{99m}Tc-Flutamide complexes



Radiolabeling studies

During year 3, we were able to optimize the labeling method of the ligand linked flutamide compounds with $^{99m}Tc(CO)_3$. We were able to setup general parameters and methods to probe the stability of the ^{99m}Tc complexes. Focus on decomposition of the compound and dissociation of the ligand from the $M(CO)_3$ center were able to determine stability of the . The key factors identified during the investigation of the complexes were the pH of the labeling solution, length of reaction time, and temperature of the solution. These parameters allowed us to investigate the stability of the flutamide portion of the molecule, as well as the complex. The tridentate ligands and 2+1 had similar preparation results, however, the stability results differed. The temperature and serum stability studies revealed the tridentate ligand remained stable throughout the study, while the 2+1 were evaluation method to evaluation the serum stability of the compounds was developed and implemented. The ^{99m}Tc complexes of the tridentate ligands (histidine and cysteine) remained intact during the course of the study, while the “2+1” approach revealed the dissociation of the imidazole flute from the complex.

In vitro and Cell binding assays

The hurdle involving the transportation of the ^{99m}Tc samples was overcome by approval from radiation safety office. This permitted method development for in vitro

testing of the ^{99m}Tc compounds with cells. The first generation of ^{99m}Tc flutamide complexes was examined with prostate cancer cells. The tridentate analogs (histidine and cysteine) were incubated with AR positive cells (DU-145) and AR negative (PC-3). The compounds illustrated some binding to the AR + cells (~2%). Although the percent binding was significantly lower than anticipated, it paved the road for further investigations with additional generation compounds.

- **Alternative approaches**

During year three, additional approaches were investigated to couple flutamide derivatives with new ligand systems. New methods were investigated on a fundamental level prior to functionalization with the biotargeting agent. As mentioned in year 2, a modified version of 2+1 approach, where two bidentate ligands were combined to form a tridentate ligand. The paper was accepted and printed in Inorganic Chemistry (See attached). Additional methods using amines and imidazole ligands utilizing a similar approach were also investigated. Synthetic methods are currently being explored to implement this methodology to functionalize with flutamide derivatives based on the current results of the first generation compounds.

The 2+1 approach was initially investigated for coupling the $Tc(CO)_3$ to a multi-modality compound for increased binding. The goal was to incorporate two flutamide targeting molecules into the compound structure. The model compounds (benzyl) illustrated the potential for preparing the flutamide compound. Experimental details are noted in the Corrected proof of the submitted article to Inorganic Chimica Acta. Although this method was believed to enhance binding, the poor stability of the 2+1 approach based on the serum and stability data limited this potential application.

Utilizing the chemistry developed for the cysteine flutamide derivatives, a new tridentate ligand was prepared by reacting cysteine with Bromomethylpyridine to generate an S functionalized S-methylpyridyl-cysteine. The ligand was found to be more effective at labeling $Tc(CO)_3$ at 10^{-6} M than the ethylene version identified in year 1.[4] The preliminary data involving the ligand and complexation with Re/^{99m}Tc$(CO)_3$ is currently being complied into a publication.

Future direction

The successful preparation of the first generation compound illustrate the capability to specifically form the desired ^{99m}Tc flutamide compounds. Although the

specific binding of these compounds was less than desirable, it does provide opportunities for improvement based on the current results. Two approaches will be examined to improve the affinity of the compounds.

1) Investigate the length of the linker to improve affinity

The 1st generation compounds, while successful at achieving the goal of preparation, may have the AR binding motif and the Tc complex to close together for effective binding. Computation modeling of the receptor and flutamide binding will be further utilized to determine the effective linker distance between the Tc complex and the flutamide binding moiety. Synthetic strategies developed for the first generation should provide adequate experience to generate a linker longer component.

2) Improve the flutamide binding moiety.

Enhancing the activity of the second generation compounds is a main priority. Modified version of generation one compounds are being adjusted to mimic more active versions of AR antagonists. The design of the next wave of compounds will follow a structure activity relationship study to provide additional information about affinity. The next prepared compounds would more closely emulate the activated version of flutamide, Hydroxyflutamide, for improve binding. The inclusion of the alpha methyl and hydroxyl group should increase the affinity of the compound to the AR. Other approaches to improve activity would involve mimicking analogous inhibitor such as bicalutamide for improved affinity that have a similar structure as the developed flutamide complexes.

Key Research Accomplishments

***Successful preparation and in vitro evaluation of the first generation of flutamide compounds of with both Re and ^{99m}Tc**

***Successful development/reporting a new labeling strategy for in situ ligand formation for the formation of tridentate ligands.**

***Successful development a new labeling strategy for in situ ligand formation for the formation of Cys pyridine.**

Reportable Outcomes

Manuscripts

1) Benny, Paul D.; Fugate, Glenn A.; Barden, Adam O.; Morley, Jennifer E.; Silva-Lopez, Elsa; Twamley, Brendan. Metal-Assisted In Situ Formation of a Tridentate

Acetylacetone Ligand for Complexation of fac-$Re(CO)_3^+$ for Radiopharmaceutical Applications. Inorganic Chemistry (2008), 47(7), 2240-2242.

2) He, Haiyang; Morely, Jennifer E.; Silva-Lopez, Elsa; Bottenus, Brienne; Montajano, Maribel; Fugate, Glenn A.; Twamley, Brendan; Benny, Paul D.. Synthesis and Characterization of Nonsteroidal-Linked $M(CO)_3^+$ (M = ^{99m}Tc, Re) Compounds Based on the Androgen Receptor Targeting Molecule Flutamide. Bioconjugate Chemistry IN PRESS

3) Benny, Paul D.; Fugate, Glenn A.; Morley, Jennifer E.; Twamley, Brenden; Trabue, Steven Synthesis and Characterization of 2,5-bis(benzyl thio)-1,3,4-thiadiazole Complexes with *fac*-$ReBr_3(CO)_3^{2-}$ *Inorganica Chimica Acta* In press

Presentations

1) Stanford, Molecular Imaging Group, Palo Alto, CA (9/9/2008)
2) Probe development Meeting, University of California- San Francisco (9/10/2008)
3) *The Cure Start Here*, Symposium for Medical Isotope Application, Radiochemistry at Washington State University. Kennewick, WA. (3/26/2008)
4) The Idaho Society of Radiology Technologists, Lewiston, ID. Future Directions in Prostate Cancer Imaging. (4/18/2008)

Conclusions

The results presented here for year three illustrate potential viability of the compounds for prostate cancer. The third year research efforts have established several important milestones to measure the success of the project. Several key preparation and evaluation of the compounds were investigated. This provided important evidence indicating the stability and the activity of the compounds. The studies in the third year established the desired ^{99m}Tc flutamide compounds can be formed and are stable in biological conditions. The studies also revealed important stability information about the 2+1 approach for flutamide analogs. Although the first generation compounds had a low affinity for the AR in prostate cells, the studies conducted during year three have provided the necessary insight in the synthesis of new analogs and preparation of the ^{99m}Tc complexes required to develop additional models to target the AR.

References

[1]Pak, Jae Kyoung; Benny, Paul; Spingler, Bernhard; Ortner, Kirstin; Alberto, Roger. Chemistry--A European Journal (2003), 9(9), 2053-2061.

[2]van Staveren, Dave R.; Benny, Paul D.; Waibel, Robert; Kurz, Philipp; Pak, Jae-Kyoung; Alberto, Roger. Helvetica Chimica Acta (2005), 88(3), 447-460.

[3]Schibli, Roger; Schwarzbach, Rolf; Alberto, Roger; Ortner, Kirstin; Schmalle, Helmut; Dumas, Cecile; Egli, Andre; Schubiger, P. August. *Bioconjugate Chemistry* **2002**, 13(4), 750-756.

[4]Fish, Windle; Gafield, Scherer; *Inorg. Chem.,* **1973**, 855-859.

Appendices

List of Personnel supported by this funding

Personnel	Position
Paul Benny	Professor, director of research
Hiayang He	Postdoctoral Research Associate

Inorganic Chemistry
including bioinorganic chemistry

Communication

Metal-Assisted In Situ Formation of a Tridentate Acetylacetone Ligand for Complexation of *fac*-Re(CO) for Radiopharmaceutical Applications

Paul D. Benny, Glenn A. Fugate, Adam O. Barden, Jennifer E. Morley, Elsa Silva-Lopez, and Brendan Twamley

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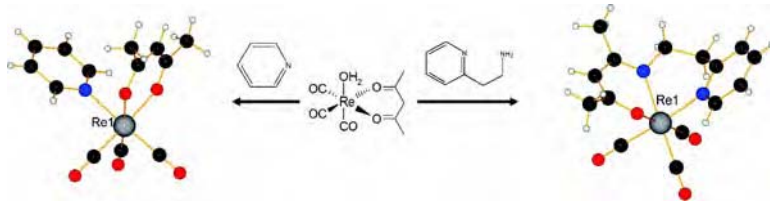

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Metal-Assisted In Situ Formation of a Tridentate Acetylacetone Ligand for Complexation of *fac*-$Re(CO)_3^+$ for Radiopharmaceutical Applications

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Reaction of $[NEt_4]_2[ReBr_3(CO)_3]$ with 2,4-pentanedione (acac) yields a complex of the type *fac*-$Re(acac)(OH_2)(CO)_3$ (**1**) under aqueous conditions. **1** was further reacted with a monodentate ligand (pyridine) to yield a *fac*-$Re(acac)(pyridine)(CO)_3$ complex (**2**). Complex **1** was found to react with primary amines to generate a Schiff base (imine) in aqueous solutions. When a mixed-nitrogen donor bidentate ligand, 2-(2-aminoethyl)pyridine, that has different coordination affinities for *fac*-$Re(acac)(OH_2)(CO)_3$ was utilized, a unique tridentate ligand was formed in situ utilizing a metal-assisted Schiff base formation to yield a complex *fac*-$Re(CO)_3$(3[(2-phenylethyl)imino]-2-pentanone) (**3**). Tridentate ligand formation was found to occur only with the Re-coordinated acac ligand. Reactions of acac with *fac*-$Re(CO)_3Br$(2-(2-aminoethyl)pyridine) (**4**) or a mixture of $[NEt_4]_2[ReBr_3(CO)_3]$, acac, and 2-(2-aminoethyl)pyridine did not yield the formation of complex **3** in water.

Technetium-99m ($t_{1/2}$ = 6.02 h; γ = 140 keV) is the radionuclide of choice in hospitals comprising 90% of all nuclear medicine imaging scans.[1] Development of organometallic technetium complexes, such as *fac*-$[^{99m}Tc(OH_2)_3(CO)_3]^+$, has provided new avenues of complex formation for diagnostic imaging.[2,3] Current labeling strategies include incubation of monodentate, bidentate, tridentate, or combination (2 + 1) ligand systems with the *fac*-$[^{99m}Tc(OH_2)_3(CO)_3]^+$ moiety.[4,5] Some of the best ligand systems (histidine, cysteine, and 2,3-diaminopropanoic acid) form tridentate complexes at >90% at 10^{-6} M.[6–9] However, complex formation below 10^{-6} M appears limited by the thermodynamics of ligand substitution of the technetium(I) center.[10]

Interest in developing new modes of complex formation has led us to investigate 2,4-pentanedione or acetylacetone (Acac) as a potential ligand system. acac is a well-established bidentate ligand that coordinates a number of transition metals. Acac can also be synthetically modified to incorporate a linked biotargeting moiety at carbon C1 and/or C3. The Schiff base or imine versions of acac are prepared by reacting a primary amine with the ligand in organic solvents. The stability of the Schiff base ligand in water may be limited by the hydrolytic nature of the imine bond. The mixed-donor (O, N) acac-derived Schiff base ligand provides an excellent ligand for rhenium with improved stability over the acac ligand alone.[11,12]

Acac-based complexes were prepared and characterized with natural rhenium to better understand the chemistry that would be potentially found with radioactive ^{99m}Tc and $^{186/188}Re$ analogues used in nuclear medicine. The reactions reported within were prepared in water to simulate reaction conditions that would be potentially translatable to the analogous radioactive complexes. The rhenium acac complex can be formed by heating $[NEt_4]_2[ReBr_3(CO)_3]$ with acac at 70 °C for 2 h in 10.0 mL of water to yield *fac*-$Re(acac)OH_2(CO)_3$ (**1**; Scheme 1). The product, **1**, remains quite soluble in water but can be isolated as a colorless solid

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(1) Jurisson, S.; Lydon, J. *Chem. Rev.* **1999**, *99*, 2205–2218.
(2) Alberto, R.; Schibli, R.; Egli, A.; Schubiger, A.; Abram, U.; Kaden, T. *J. Am. Chem. Soc.* **1998**, *120*, 7987–7988.
(3) Alberto, R.; Ortner, K.; Wheatley, N.; Schibli, R.; Schubiger, A. *J. Am. Chem. Soc.* **2001**, *123*, 3135–3136.
(4) Alberto, R.; Pak, J.; van Staveren, D.; Mundwiler, S.; Benny, P. *Biopolymers* **2004**, *76*, 324–333.
(5) Mundwiler, S.; Kundig, M.; Ortner, K.; Alberto, R. *Dalton Trans.* **2004**, *9*, 1320–1328.
(6) Pak, J.; Benny, P.; Spingler, B.; Ortner, K.; Alberto, R. *Chem.—Eur. J.* **2003**, *9*, 2053–2061.
(7) Liu, Y.; Pak, J.; Schmutz, P.; Bauwens, M.; Mertens, J.; Knight, H.; Alberto, R. *J. Am. Chem. Soc.* **2006**, *128*, 15996–15997.
(8) van Staveren, D.; Benny, P.; Waibel, R.; Kurz, P.; Pak, J.; Alberto, R. *Helv. Chim. Acta* **2005**, *3*, 447–460.
(9) Mindt, T.; Struthers, H.; Brans, L.; Anguelov, T.; Schweinsberg, C.; Maes, V.; Tourwe, D.; Schibli, R. *J. Am. Chem. Soc.* **2006**, *47*, 15096–15097.
(10) Grundler, P.; Helm, L.; Alberto, R.; Merbach, A. *Inorg. Chem.* **2006**, *45*, 10378–10390.
(11) Benny, P.; Green, J.; Engelbrecht, H.; Barnes, C.; Jurisson, S. *Inorg. Chem.* **2005**, *7*, 2381–2390.
(12) Benny, P.; Barnes, C.; Piekarski, P.; Lydon, J.; Jurisson, S. *Inorg. Chem.* **2003**, *20*, 6519–6527.

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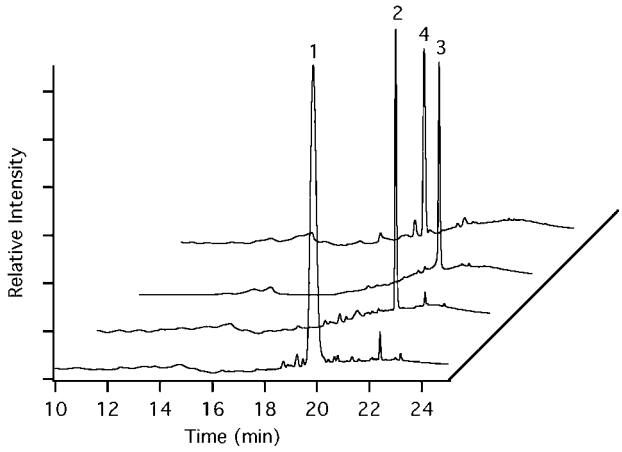
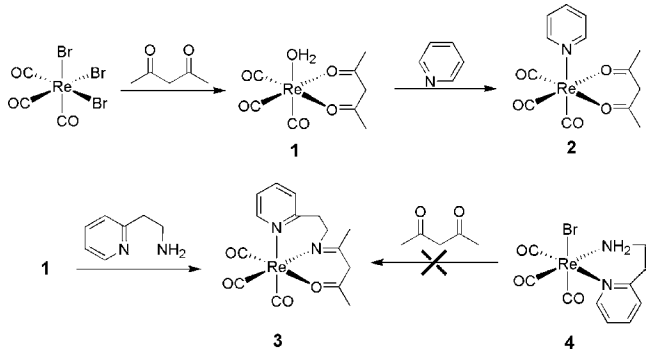


Figure 1. UV−HPLC trace at 220 nm of **1**−**4**.

Scheme 1. Synthetic Route for Preparation of Rhenium acac Complexes and Subsequent Reactions with Mono- and Bidentate Ligands To Yield "2 + 1" and in Situ Formed Tridentate Complexes



in high yield through concentration and cooling of the solution in a refrigerator (∼2 °C) overnight. High-performance liquid chromatography (HPLC) studies of the solution and the isolated solid revealed a single peak of complex **1** at 20.6 min verified by NMR (Figure 1).

Complex **1** is a versatile reagent because it can be isolated as a solid or utilized directly from the reaction mixture. The addition of a second monodentate ligand to **1** generated a "2 + 1" style of complex. When **1** is reacted with pyridine at 70 °C overnight, the complex *fac*-Re(acac)(CO)$_3$py (**2**) can be prepared in high yield. **2** is the only product observed from the solution. Even in the presence of excess pyridine, displacement of the acac ligand was not observed. The formation of **2** can be observed by the appearance of a new peak at 22.3 min in the HPLC (Figure 1). The X-ray structure of **2** was obtained by diffusion of pentane into a dichloromethane solution of **2** (Figure 2).[13] The octahedral complex has comparable Re−CO bonds (1.89–1.92 Å) with an asymmetric axis elongated along the Re−pyr (Re1−N1 2.20 Å) and acac (Re−O1 or Re−O2 2.12 Å) axes. The acac ligands show a minimally constrained bite angle (O1−Re−O2 85.07°). The pyridine N1 is equidistant to the O1/O2 of the acac ligand (O−Re−N1 82.67–83.3°).

(13) **2**, monoclinic space group *P*2(1)/*c* with cell dimensions $a = 14.9940(7)$ Å, $b = 6.8687(3)$ Å, and $c = 14.1746(6)$ Å and $\beta = 104.698(1)°$.

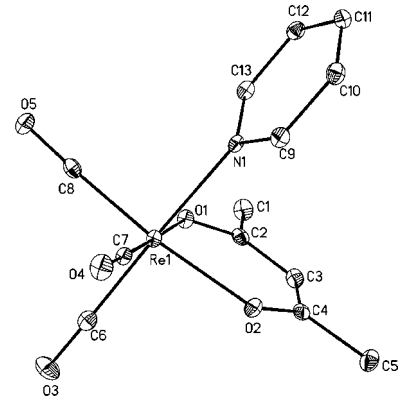


Figure 2. Molecular structure of **2** (thermal displacement 30%). Hydrogen atoms are omitted clarity. Bond distances (Å): Re1−O1 2.1189(19), Re1−O2 2.1226(19), Re1−C6 1.926(3), Re1−C7 1.896(3), Re1−C8 1.903(3), Re1−N1 2.209(2). Bond angles (deg): O1−Re−O2 85.07(8), O1−Re−C6 95.29(10), O1−Re−C7 177.72(10), O1−Re−C8 92.76(10), O2−Re−N1 82.67(8), O2−Re−C6 95.62(11), O2−Re−C7 94.55(10), O2−Re−C8 174.08(10), N1−Re−C6 177.88(10), N1−Re−C7 94.39(10), N1−Re−C8 91.62(11).

Although the "2 + 1" complex **2** is coordinatively saturated, ligand displacement may limit the effectiveness of the complex in vivo. The formation of a tridentate ligand compared to the "2 + 1" complex may have increased stability toward substitution because of the chelate effect. The "2 + 1" complex of **2** can be transformed by an in situ reaction into a tridentate complex utilizing the reactivity of acac in **1** to form an imine from a primary amine. An analogous rhenium pyridine aldehyde complex was previously demonstrated to form a bidentate imine complex system from a primary amine; however, the bidentate complex had limited stability toward ligand substitution.[14,15] *fac*-Re(CO)$_3$(3[(2-phenylethyl)imino]-2-pentanone)] (**3**) was prepared in a two-step process: formation of the acac complex **1** followed by the addition of a second bidentate ligand (Scheme 1). Complex **3** was formed either stepwise or as a single-pot reaction. The formation of the imine bond in **3** was observed by the addition of 2-(2-aminoethyl)pyridine to an aqueous solution of complex **1** followed by heating at 70 °C. The product precipitated as a colorless solid upon cooling to room temperature. The reaction progress was monitored by HPLC, where the disappearance of **1** and the appearance of **3** at 21.4 min were observed in the chromatogram (Figure 1). Crystals of **3** were obtained by slow evaporation of a methanol/water solution at room temperature (Figure 3).

The solid-state structures of the "2 + 1" complex **2** and the tridentate complex **3** have many structural similarities in bond distances (i.e., Re−pyridine ∼2.2 Å, Re−O1 2.13 Å, Re−CO ∼1.9 Å) and angles (O1−Re−N1 82.24(8)° in **3** has a bite angle similar to that of the acac ligand in **2**). However, the methylene carbons (C6 and C7) have larger than typical bond angles (113–115°). C3 of the acac

(14) Wang, W.; Spingler, B.; Alberto, R. *Inorg. Chim. Acta* **2003**, *355*, 386–393.

(15) Alvarez, C.; Garcia-Rodriguez, R; Miguel, D. *Dalton Trans.* **2007**, *32*, 3546–3554.

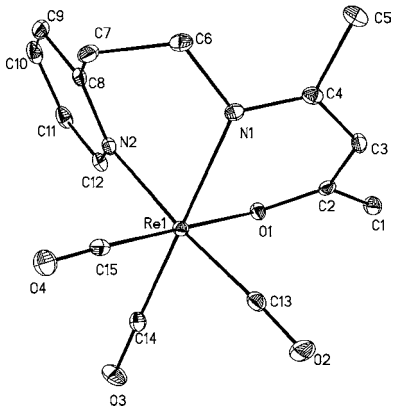


Figure 3. Molecular structure of **3** (30% thermal displacement). Hydrogen atoms are omitted for clarity. Bond distances (Å): Re1−O1 2.1336(18), Re1−N1 2.166(2), Re1−N2 2.197(2) Re1−C13 1.925(3), Re1−C14 1.933(3), Re1−C15 1.901(3). Bond angles (deg): O1−Re−N1 82.24(8), O1−Re−C13 93.27(10), O1−Re−C14 92.46(9), O1−Re−C15 178.39(9), N1−Re−N2 80.36(8), N1−Re−C13 96.05(10), N1−Re−C14 173.77(9), N1−Re−C15 99.16(10), N2−Re1−C13 175.54(10), N2−Re1−C14 95.85(10), N2−Re1−C15 95.76(10).

ligand in **3** is also positioned slightly out of plane because of the steric restraints of the linked pyridine and the imine bond of the tridentate system in **3** compared to **2**.

The Schiff base formation of the tridentate complex utilizes distinct differences in the coordination strength of the bidentate ligand 2-(2-aminoethyl)pyridine, containing a primary amine and an aromatic amine. It is proposed that the pyridine ligand, as opposed to the amine, first coordinates to the rhenium center, replacing the labile aqua ligand. The uncoordinated amine donor is available for nucleophilic attack on the C2 of the coordinated acac ligand. The coordinated oxygen from the acac ligand is converted to water during the Schiff base condensation and probably remains coordinated for a brief moment prior to displacement by the more favorable imine donor from the tridentate ligand. Reactivity of the amine donor with the acac ligand is believed to depend on the effective chelate ring size and steric constraints of the number of methylene carbons between the pyridine and amine.

Displacement of the acac ligand from complex **1** upon introduction of 2-(2-aminoethyl)pyridine was a primary concern. The potential displacement byproduct *fac*-Re(2-(2-aminoethyl)pyridine)$Br(CO)_3$ (**4**) was prepared by refluxing $[NEt_4]_2[ReBr_3(CO)_3]$ with 2-(2-aminoethyl)pyridine in methanol (Scheme 1). HPLC of the reaction yielded a single peak at 20.0 min corresponding to **4**. The complex was characterized and utilized as a reference for HPLC comparison (Figure 1). Single crystals were obtained from a methanol solution of **4** at 0 °C after several days (Figure 4). **4** was further evaluated to determine the dissociation/reactivity of the coordinated 2-(2-aminoethyl)pyridine by introducing excess acac ligand in water (Scheme 1). A second slightly more hydrophilic peak was observed in HPLC over a prolonged period, which may be due to substitution of the coordinated

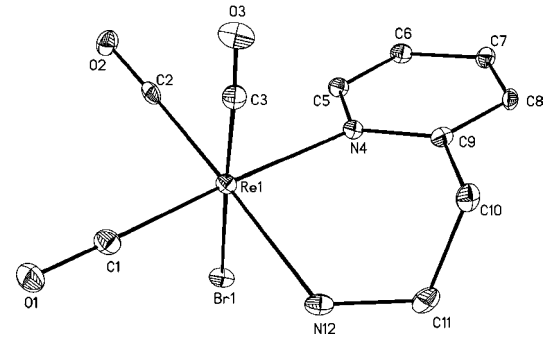


Figure 4. Molecular structure of **4** (thermal displacement 30%). The solvent molecule and hydrogen atoms are omitted for clarity. Bond distances (Å): Re1−Br1 2.1189(19), Re1−O2 2.1226(19), Re1−C6 1.926(3), Re1−C7 1.896(3), Re1−C8 1.903(3), Re1−N1 2.209(2). Bond angles (deg) O1−Re−O2 85.07(8), O1−Re−C6 95.29(10), O1−Re−C7 177.72(10), O1−Re−C8 92.76(10), O2−Re−N1 82.67(8), O2−Re−C6 95.62(11), O2−Re−C7 94.55(10), O2−Re−C8 174.08(10).

bromine in **4** with water. Under the conditions examined, neither the formation of **3** or the displacement of 2-(2-aminoethyl)pyridine by acac yielding **1** was observed, suggesting that 2-(2-aminoethyl)pyridine remains coordinated to the rhenium center in **4** without dissociation or activation of the complex toward Schiff base formation. Although free ligand formation is possible, we examined the possibility that the ligand could be formed in situ by the addition of acac and 2-(2-aminoethyl)pyridine in water followed by the addition of *fac*-$[ReBr_3(CO)_3]^{2-}$. The mixture yielded **4** or the aquo-coordinated complex as observed by HPLC, and no formation of the tridentate ligand complex **3** was observed.

In conclusion, we have demonstrated that acac can be utilized as a bidentate ligand system in a "2 + 1" approach or utilizing coordination differences to generate a tridentate ligand system while coordinated to the rhenium metal center. This new methodology has the potential for linking to targeting molecules, such as small molecules, peptides, and antibodies, for generating in situ tridentate complexes for nuclear medicine applications.

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Supporting Information Available: Full syntheses, characterization of compounds, and X-ray crystallographic bond angles and distances tables (PDF) and X-ray structural information for **2**−**4** (CIF). This material is available free of charge via the Internet at http://pubs.acs.org.

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Article

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Synthesis and Characterization of Nonsteroidal-Linked $M(CO)_3^+$ ($M = {}^{99m}Tc$, Re) Compounds Based on the Androgen Receptor Targeting Molecule Flutamide

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Androgen receptors are overexpressed in most primary and metastatic prostate cancers. A series of single photon emission computed tomography imaging agents (SPECT) utilizing the organometallic radioactive imaging species, *fac*-$^{99m}Tc(OH_2)_3(CO)_3^+$, were prepared on the basis of the structure of Flutamide, a potent nonsteroidal antiandrogen prostate cancer drug. Novel bifunctional chelate-linked Flutamide analogues were prepared using a newly developed universal alkylating reagent, 2-bromo-*N*-[4-nitro-3-(trifluoromethyl)phenyl]-acetamide, **1**. From compound **1**, several ligands (i.e., cysteine **2**, histidine **5**, imidazole **3**) were conjugated to the flutamide derivative to yield targeting ligands capable of either tridentate or monodentate coordination in a "2 + 1" complex. *fac*-$Re(CO)_3^+$ complexes were prepared and characterized with the functionalized conjugates to yield *fac*-$Re(CO)_3$(2-amino-3-(1-(2-(4-nitro-3-(trifluoromethyl)phenylamino)-2-oxoethyl)-1*H*-imidazol-4-yl) propanoate), **4**, *fac*-Re $(CO)_3$(2-(*S*-cysteinyl)-*N*-[4-nitro-3-(trifluoromethyl) phenyl]-acetamide), **6**, and *fac*-$Re(CO)_3$(picolinate)(2-(1*H*-imidazol-1-yl)-*N*-[4-nitro-3-(trifluoromethyl)phenyl]-acetamide), **7**. The corresponding radioactive ^{99m}Tc analogues were prepared and stability studies of the radioactive compounds were also conducted.

INTRODUCTION

Prostate cancer is the second leading cancer affecting men in the United States (*1*). Current screening methods utilized in medicine for prostate cancer involve a digital rectal exam, prostate specific antigen (PSA), and/or prostate core biopsies (*2, 3*). Each of these methods has their own limitations in specificity and accuracy in diagnosing prostate cancer. Major limitations of current diagnostic methods are caused by the inability to noninvasively image and spatially resolve cancerous tissues amidst normal prostate tissue without perturbation of the organ. Prostate cancer cells have been found to overexpress the androgen receptor (AR) during the cancer development (*4*). The AR provides an excellent biological target for discriminating from normal prostate cells due to the increased up-regulation of the AR. The overexpression of AR is most likely due to the increased transportation of testosterone into the cancerous cells, which has been attributed to a role in DNA transcription and cell proliferation (*5*).

Current treatment of prostate cancer involves single or combination treatment strategies from hormone treatment, radiation implants, surgical removal of the prostate, and/or chemical castration for eliminating testosterone production. Major side effects of the treatment methods involve decrease or elimination of normal sexual function and loss of urinary function. Hormone therapy, targeting the androgen receptor, is typically regarded as the first round of treatment for prostate cancer to control the prostate size and tumor growth. Several nonsteroidal antiandrogens, such as nilutamide, bicalutamide, flutamide, and the corresponding metabolite hydroxyflutamide, have been utilized as hormonal therapeutic agents in a clinic setting for a number of years (Figure 1) (*6*−*11*). Although hormone therapy may have some initial success, recurrent or hormone refractory prostate cancers may occur 1−3 years after hormone treatment as an advanced form of metastatic cancer that is resistant to chemotherapy and is believed to be androgen-independent due to a mutation of the AR (*12*).

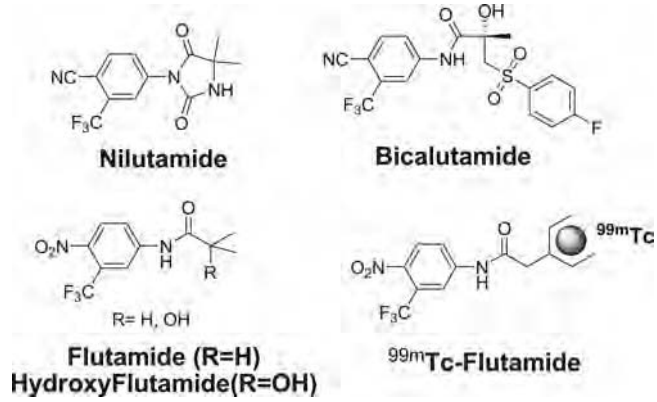

Figure 1. Examples of nonsteriodal antiandrogens clinically utilized in hormone therapy for prostate cancer and the ^{99m}Tc complex-linked flutamide reported within.

The presence or absence of AR in prostate cancer could be identified by a noninvasive imaging probe and would provide a key indicator in early screening as well as disease progression. Several positron emission tomography (PET) AR targeting analogues (i.e., flutamide, testosterone, dihydrotestosterone) have been previously reported for imaging. However, limitations in the half-life of positron (β^+) emitting isotopes, production, and PET imaging instrumentation required may have restricted clinical applications (*13*−*17*). Albeit with decreased resolution compared to PET, the development of a clinically routine single photon emission computed tomography (SPECT) imaging agent

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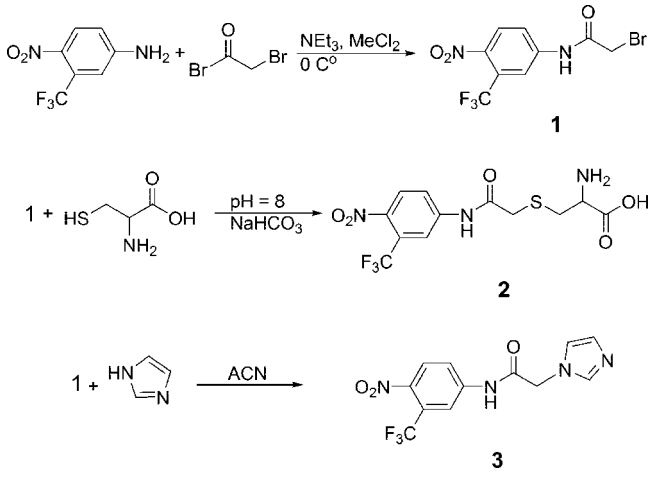
Scheme 1. Preparation of 2-Bromo-*N*-[4-nitro-3-(trifluoromethyl)phenyl]acetamide, 1, and the Corresponding Ligand-Linked Flutamide Compounds by Conjugation of 1 with Cysteine and Imidazole to Generate the Respective Compounds 2 and 3



utilizing ^{99m}Tc ($E_\gamma = 140$ keV, $I_\gamma = 89\%$, $t_{1/2} = 6.02$ h) would provide an alternative for hospitals already equipped with a SPECT camera. A number of small-molecule-linked ^{99m}Tc(V) oxo complexes with steroids, such as testosterone, have been reported in the literature for prostate imaging (*18, 19*). The recent development of the organometallic precursor, *fac*-^{99m}Tc-$(OH_2)_3(CO)_3^+$, has several important advantages over Tc(V) oxo complexes for biological targeting of prostate cancer (*20*−*24*). The *fac*-^{99m}Tc$(OH_2)_3(CO)_3^+$ species can be conveniently prepared in aqueous conditions from pertechnetate isolated from a ^{99}Mo/^{99m}Tc generator with an Isolink kit produced by Tyco (*25*). The ^{99m}Tc organometallic complexes have a reduced molecular weight, molecular volume, polarity, and ligand size compared the ^{99m}Tc(V) oxo complexes. The stable CO ligands also help increase the lipophilicity of the compounds, which may assist in hepatic clearance rather than renal clearance. The chemical and structural differences of the two types of Tc compounds can have important implications in biological interactions of complexes. This is of particular importance, as bladder collection of more polar ^{99m}Tc agents would significantly impact the clarity of the images by increasing the background or completely obstructing the prostate.

Our investigation reported herein was designed to develop a model system to explore the feasibility of ^{99m}Tc-linked flutamide derivatives that utilized the *fac*-^{99m}Tc$(OH_2)_3(CO)_3^+$ precursor. We developed a central strategy that would generate a library of flutamide analogues capable of complexing the technetium species. The universal flutamide derivative was prepared and conditions determined to use general alkylation methods to covalently link a number of ligand systems to the flutamide derivative (Scheme 1). The flutamide-linked ligands were examined with both the nonradioactive rhenium for chemical and structural characterization followed by the radioactive ^{99m}Tc analogues to determine labeling effectiveness and radiochemical stability.

EXPERIMENTAL SECTION

All reagents and organic solvents were purchased from Aldrich, Acros, or Fluka in reagent grade or better and were used without further purification. Rhenium starting materials $Re(CO)_5OTf$, and *fac*-$[Re(CO)_3(H_2O)_3]OTf$, were prepared by literature methods from $Re_2(CO)_{10}$ purchased from Strem (*24, 26*). Rhenium complexes *fac*-$Re(CO)_3$(Histidine) and *fac*-$Re(OH_2)(CO)_3$(picolinate) were prepared by reacting the rhenium starting materials with the appropriate ligand according to reported methods (*27, 28*). Normal mouse serum (L10410) with 0.1% sodium azide was purchased from Caltag Laboratories and was centrifuged to remove particulate matter prior to use. Elemental analyses were performed by Quantitative Technologies, Inc., New Jersey. Separation and identification of compounds were conducted on a Perkin-Elmer Series 200 high pressure liquid chromatograph (HPLC) equipped with a UV/vis series 200 detector and a Perkin-Elmer Radiomatic 610TR detector utilizing a 30 cm 5 μm Agilent Zorbex SB-C18 column. The compounds were separated with a reverse-phase gradient system beginning with 0.1% trifluoroacetic acid (TFA) aqueous eluent gradually shifting to methanol according to the following method, 0−3.0 min (100% TFA), 3.0−9.0 min (75% TFA, 25% MeOH), 9.0−20.0 min (25% to 100% MeOH linear gradient), 20.0−25.0 min (100% MeOH) at a flow rate of 1.0 mL/min or 5.0 mL/min for separation. ^{1}H NMR spectra were recorded on a Varian 300 MHz spectrometer, and chemical shifts were referenced to the internal standard sodium 3-(trimethylsilyl) propionate-d_4 (TSP, 0.00 ppm) in D_2O or the residual solvent signal and/or tetramethylsilane (TMS) in organic solvents. Spectra were processed using Varian VNWR 6.1 software. IR spectra were collected with a Thermo Nicolet 6700 FT-IR with an ATR cell and analyzed with OMNIC 7.1a software. Mass measurements were performed as Q3 scans on an API4000 triple quadrupole (Applied Biosystems). Sample concentrations of ~0.1 μg/μL in methanol were infused at 10 μL/min, with orifice heating on, declustering potential 20 V, and entrance potential 10 V.

2-Bromo-*N*-[4-nitro-3-(trifluoromethyl)phenyl]acetamide, 1. Et_3N (5.9 mL, 42 mmol) was added slowly to a solution of bromoacetyl bromide (8.1 g, 40 mmol) and 5-amino-2-nitrobenzotrifluroride (7.0 g, 34 mmol) in CH_2Cl_2 (200 mL) at 0 °C. The solution was stirred for 2 h at 0 °C, which resulted in a clear solution. The solution was continuously stirred and allowed to reach rt overnight. The solution was washed with 0.6 M of HCl three times (200 mL × 3), followed by water. The organic phase was dried over Na_2SO_4, filtered, and evaporated to dryness to give a pure slight yellow product (9.9 g, 89%). Anal. Calcd for $C_9H_6BrF_3N_2O_3$: C, 33.05; H, 1.85; N, 8.57. Found: C, 33.00; H, 1.62; N, 8.33. ^{1}H NMR [δ (ppm), $CDCl_3$] 8.53 (s, 1H), 8.02 (m, 3H), 4.08 (s, 2H). ^{13}C NMR [δ (ppm), $CDCl_3$] 164.5, 141.3, 127.4, 125.6 (q), 123.6, 122.7, 120.0, 118.7 (q), 29.1. MS [$M^+ - Na^+$] 349.0, 351.1.

2-(*S*-Cysteinyl)-*N*-[4-nitro-3-(trifluoromethyl)phenyl]acetamide, 2. To a solution of excess cysteine (1.21 g, 10 mmol) in water (50 mL) was added $NaHCO_3$ (10 mL, 1 M), followed by **1** (1.0 g, 3.0 mmol) in CH_2Cl_2 (50 mL). The biphasic mixture was vigorously stirred overnight at rt. A small amount off-white solid product was observed in the reaction mixture and additional material was formed after selective evaporation of CH_2Cl_2 from the mixture. The off-white solid was filtered and dried under vacuum (0.75 g, 67%). Anal. Calcd for $C_{12}H_{12}F_3N_2O_5S \cdot 0.6H_2O$: C, 38.12; H, 3.52; N, 11.11. Found: C, 37.89; H, 3.28; N, 11.38. ^{1}H NMR [δ (ppm), DCl/D_2O, 0.25 M] 7.98 (d, $J = 2.1$ hz, 1H), 7.95 (d, $J = 9.0$ hz, 1H), 7.82 (dd, $J = 2.1$ and 9.0 hz, 1H), 4.48 (dd, $J = 4$ and 8 hz, 1H), 3.70 (s, 2H), 3.46 (dd, $J = 4$ and 15 hz, 1H), 3.29 (dd, $J = 8$ and 15 hz, 1H). ^{13}C NMR [δ (ppm), DCl/D_2O, 0.25 M] 173.4, 173.1, 145.2, 145.0, 130.3, 126.6, 125.9, 121.6, 55.4, 39.2, 35.0, 29.1. MS [M^+] 367.9, [$M^+ - Na^+$] 389.9.

2-(1*H*-Imidazol-1-yl)-*N*-[4-nitro-3-(trifluoromethyl)phenyl]acetamide, 3. To an acetonitrile solution (10 mL) containing imidazole (1.57 g, 5 mmol) was added **1** (0.33 g, 1 mmol). The solution was stirred overnight at rt, then evaporated to dryness under vacuum to yield a faint yellow solid. Water (10 mL) was added to the solid and the solution filtered. HCl (0.5 M, 10

mL) was added to the collected solid, and the corresponding mixture was centrifugated, and the solution collected, and the solid discarded. The slow addition of $NaHCO_3$ (1 M, 4 mL) to the centrifuged solution caused the formation of a yellow precipitate, which was filtered and dried to yield the desired faint yellow product **3** (0.26 g, 82%). Anal. Calcd for $C_{12}H_9F_3N_4O_3$: C, 45.87; H, 2.89; F, 18.14; N, 17.83; O, 15.28. Found: C, 44.60; H, 2.84; N, 17.07. ^{1}HNMR [δ (ppm), methanol-d_4] 9.04 (t, 1H), 8.23 (d, 1H), 8.03 (m, 2H), 7.69 (t, 1H), 7.27 (t, 1H), 5.36 (s, 2H). ^{13}CNMR [δ (ppm), methanol-d_4] 166.8, 142.9, 138.3, 127.0, 126.9, 124.5, 124.0, 122.5, 121.1, 117.9, 117.8, 49.5. MS [M^+] 315.0, [$M^+ - Na^+$] 337.0.

***fac*-Re(CO)$_3$(2-amino-3-(1-(2-(4-nitro-3-(trifluoromethyl)phenylamino)-2-oxoethyl)-1*H*-imidazol-4-yl)propanoate), 4.** To a room temperature acetonitrile solution (80 mL) of *fac*-$Re(CO)_3$(Histidine) (0.84 g, 2 mmol) and **1** (0.72 g, 2.2 mmol) was added Cs_2CO_3 (0.60 g, 2.2 mmol) and stirring continued overnight. The solution was filtered to remove the residual Cs_2CO_3 and salt byproducts, and the filtrate was collected and evaporated to dryness. The solid residue obtained from the filtrate was washed with a minimal amount of ethanol and water and dried under vacuum to yield the desired faint yellow product **4** (1.2 g, 90%). Anal. Calcd for $C_{18}H_{13}F_3N_5O_8Re$: C, 32.24; H, 1.95; N, 10.44. Found: C, 31.81; H, 2.17; N, 9.96. ^{1}H NMR [δ (ppm), acetone-d_6] 11.90 (s, 1H), 8.26 (d, 1H, J = 1.8 Hz), 8.12 (m, 3H), 7.18 (s, 1H), 5.75 (dd, J = 11.4 and 6.0 Hz, 1H, 1H), 5.4 (d, J = 17.1 Hz, 1H), 4.35 (overlapped, 1H), 4.34(overlapped, 1H), 3.51 (dd, J = 13.5 and 3 Hz, 1H), 3.37 (dd, J = 13 and 3 Hz, 1H). ^{13}C NMR [δ (ppm), acetone-d_6] 197.9, 196.5, 183.9, 165.8, 143.9, 142.9, 142.5, 134.9, 127.6, 124.4, 124.2, 123.9, 122.3,120.8,117.5, 52.4, 49.1. IR [cm^{-1}] 2018, 1879, 1604, 1525, 1342, 1147. MS [M^+] 672.3, [$M^+ - Na^+$] 694.2.

2-Amino-3-(1-(2-(4-nitro-3-(trifluoromethyl)phenylamino)-2-oxoethyl)-1*H*-imidazol-4-yl)propanoic Acid, 5. To an acetone solution (15 mL) containing compound **4** (0.34 g, 0.5 mmol) was added HCl solution (15 mL, 1 M), followed by H_2O_2 (0.6 mL) at rt. The mixture was continuously stirred and monitored by HPLC to determine reaction progress. After 3 d, the starting material **4** was no longer observed in the chromatogram, indicating the complete conversion of the Re starting material to the ligand **5**. The reaction mixture was neutralized by Na_2CO_3 (1 M), and the solution was directly purified by preparative HPLC to yield the yellow product **5** as a semisolid. (0.169 g, 83%). Anal. Calcd for $C_{19}H_{18}F_9N_5O_{10}$: C, 35.25; H, 2.80; F, 26.41; N, 10.82; O, 24.71. 35.29, H, 2.70 N, 9.35. ^{1}H NMR [δ (ppm), methanol-d_4] 9.14 (s, 1H), 8.30 (s, 1H), 8.06 (m, 2H), 7.74 (s, 1H), 5.43 (s, 2H), 4.47 (dd, J = 6 and 7 Hz, 1H), 3.56 (dd, J = 6 and 16 Hz, 1H), 3.46 (dd, J = 7 and 16.0 Hz, 1H). ^{13}C NMR [δ (ppm), methanol-d_4] 168.9, 165.3, 143.1, 142.6, 137.5, 127.6, 127.1, 124.5, 124.0, 122.8, 120.4, 118.0, 51.7, 51.5, 25.5. MS [M^+] 402.2.

***fac*-Re(CO)$_3$(2-(S-cysteinyl)-*N*-[4-nitro-3-(trifluoromethyl)phenyl]acetamide), 6.** To a solution of **2** (0.18 g, 0.5 mmol) and $[Re(CO)_5OTf]$ (0.25 g, 0.52 mmol) in methanol (30 mL) was added Et_3N (75 μL, 0.54 mmol). The mixture was refluxed for 2 h, concentrated to 5 mL by rotary evaporation, and purified by preparative HPLC to yield the product **6** as a pale yellow solid (0.22 g, 69%). Anal. Calcd for $C_{15}H_{11}F_3N_3O_8ReS$: C, 28.30; H, 1.74; N, 6.60. Found: C, 27.99; H, 1.81; N, 6.47. ^{1}H NMR [δ (ppm), methanol-d_4, −14 °C] 8.25 (d, J = 2.1 hz, 1H), 7.98−8.09 (m, 2H), 6.24 (d, J = 10 hz, 1H), 5.02 (d, J = 10 hz, 1H), 4.36 (s, 1H), 4.24 (m, 1H), 3.92 (d, J = 15.3 Hz, 1H), 3.80 (d, J = 15.3 Hz, 1H), 3.04 (m, 2H). ^{13}C NMR [δ (ppm), methanol-d_4] 195.4, 193.3, 181.4, 167.4, 144.3, 143.9, 128.2, 125.5 (q),123.9, 121.6, 119.2 (q), 59.4, 41.7, 33.1. IR [cm^{-1}] 3000, 2029, 1903, 1530, 1267, 1146, 755. MS [M^+] 638.1, [$M^+ - Na^+$] 660.2.

Table 1. X-ray crystal data and structure refinement for compound 7

empirical formula	$C_{24}H_{19}F_3N_5O_9Re$	
formula weight	764.64	
temperature	90(2) K	
wavelength	0.71073 Å	
crystal system	monoclinic	
space group	*C*2/*c*	
unit cell dimensions	a = 19.0902(11) Å	α = 90°.
	b = 14.6852(8) Å	β = 100.5910(10)°.
	c = 20.6888(12) Å	γ = 90°.
volume	5701.2(6) Å^3	
Z	8	
density (calculated)	1.782 mg/m^3	
absorption coefficient	4.341 mm^{-1}	
F(000)	2976	
crystal size	0.34 × 0.25 × 0.23 mm^3	
crystal color and habit	yellow block	
diffractometer	Bruker/Siemens SMART APEX	
θ range for data collection	1.76° to 27.50°.	
index ranges	$-24 \le h \le 24$, $-19 \le k \le 19$, $-26 \le l \le 26$	
reflections collected	42 304	
independent reflections	6552 [R(int) = 0.0260]	
completeness to θ = 27.50°	100.0%	
absorption correction	semiempirical from equivalents	
max. and min. transmission	0.367 and 0.290	
solution method	XS, SHELXTL v. 6.14 (Bruker, 2003)	
refinement method	full-matrix least-squares on F^2	
data/restraints/parameters	6552/0/384	
goodness-of-fit on F^2	1.040	
final R indices [$I > 2\sigma(I)$]	$R1$ = 0.0201, w$R2$ = 0.0478	
R indices (all data)	$R1$ = 0.0233, w$R2$ = 0.0495	
largest diff. peak and hole	1.817 and −0.533 e.Å^{-3}	

***fac*-Re(CO)$_3$(picolinate)(2-(1*H*-imidazol-1-yl)-*N*-[4-nitro-3-(trifluoromethyl)phenyl]acetamide), 7.** To a methanol solution (5 mL) of *fac*-$Re(OH_2)(CO)_3$(picolinate) (0.033 g, 0.081 mmol) was added **3** (0.028 g, 0.089 mmol), and the solution was stirred at 50 °C for 3 h. The reaction solution was evaporated to dryness, then purified by preparative HPLC to yield the product **7** as a yellow solid. (0.040 g, 70%). X-ray quality single crystals of compound **7** were isolated as faint yellow rectangular blocks by slow evaporation of the compound in a 1:1 mixture of hexane/acetone. Anal. Calcd for $C_{21}H_{13}F_3N_5O_8Re$: C, 35.70; H, 1.85; N, 9.91. Found: C, 36.15; H, 1.97; N, 9.56. ^{1}H NMR [δ (ppm), methanol-d_4] 8.97 (dt, 1H), 8.05 (m, 6H), 7.76 (m, 1H), 7.14 (t, 1H), 6.96 (t, 1H), 4.90 (s, 2H). ^{13}C NMR [δ (ppm), methanol-d_4] 196.6, 174.2, 165.9, 152.2, 149.5, 141.2, 140.5, 129.2, 128.5, 124.2, 127.1, 124.5, 124.1, 122.5, 117.8, 117.7, 49.9. IR [cm^{-1}] 2021, 1912, 1653, 1537, 1345, 1167. MS [M^+] 708.4, [$M^+ - Na^+$] 730.5.

X-ray Crystallography. Crystals of compound **7** were removed from the flask; a suitable crystal was selected, attached to a glass fiber, and data were collected at 90(2) K using a Bruker/Siemens SMART APEX instrument (Mo Kα radiation, λ = 0.71073 Å) equipped with a Cryocool NeverIce low-temperature device. Data were measured using omega scans 0.3° per frame for 5 s, and a full sphere of data were collected. A total of 2400 frames were collected with a final resolution of 0.77 Å. Cell parameters were retrieved using SMART software (*29*) and refined using SAINTPlus (*30*) on all observed reflections. Data reduction and correction for Lp and decay were performed using the SAINTPlus software. Absorption corrections were applied using SADABS (*31*). The structure was solved by direct methods and refined by least-squares method on F^2 using the SHELXTL program package (*32*). The structure was solved in the space group *C*2/*c* by analysis of systematic absences. No decomposition was observed during data collection. Details of the data collection and refinement are given in Table 1. Further details are provided in the Supporting Information.

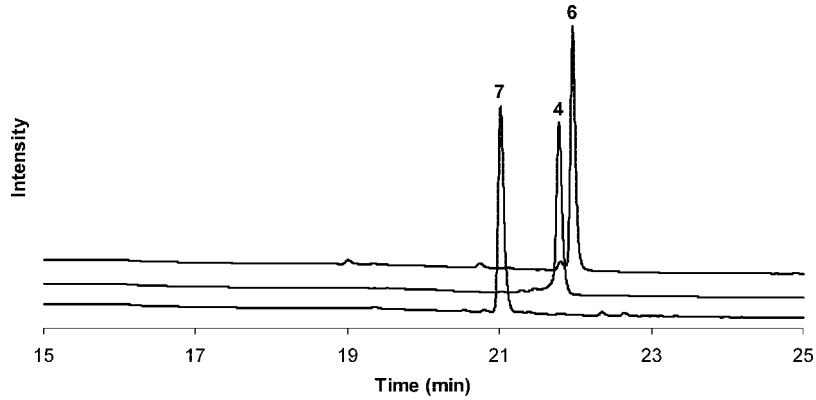


Figure 2. UV/vis HPLC (220 nm) chromatograph of Flutamide-linked rhenium tricarbonyl complexes of **4**, **6**, and **7**.

Radiochemistry. ^{99m}Tc-pertechnetate ($^{99m}TcO_4^-$) was purchased from Cardinal Health in Spokane, WA. IsoLink kits to prepare the $^{99m}Tc(H_2O)_3(CO)_3^+$ precursor were obtained as a gift from Tyco, Inc. The radiolabeled products were characterized by Perkin-Elmer Series 200 HPLC under the same mobile-phase conditions previously mentioned, except the γ emissions were detected with a Perkin-Elmer Radiomatic 610TR detector equipped with a Gamma B cell (80 μL loop). The *fac*-$^{99m}Tc(H_2O)_3(CO)_3^+$ precursor was prepared according to the manufacture's procedure, the addition via syringe of 10−20 mCi of $Na^{99m}TcO_4$ in 1 mL saline to the Isolink kit followed by heating at 95 °C for 20 min. The resulting solution was cooled in an ice bath, neutralized with HCl (∼100 μL, 1 M) and checked on the γ-HPLC system to ensure complete formation of the precursor.

General $^{99m}Tc(H_2O)_3(CO)_3^+$ Radiolabeling Procedure. The ligand **2** or **5** (100 μL, 10^{-4} or 10^{-5} M) and phosphate buffer (800 μL, 0.1 M) at pH 7.4 was added to a sealable labeling vial (5.0 mL). The vial was sealed and degassed with nitrogen for ∼10 min. The $^{99m}Tc(H_2O)_3(CO)_3^+$ precursor solution (100 μL) was added to the degassed vial and the vial heated for 30 min at 70 °C. The reaction mixture was then allowed to cool on an ice bath prior to injection and analysis by radio-HPLC.

2 + 1 Radiolabeling Procedure. *Method 1: One-Pot Preparation.* Ligand **3** (100 μL, 10^{-2} M), picolinic acid (100 μL, 10^{-4} M), and phosphate buffer (700 μL, 0.1 M) at pH 7.4 were added in order to a sealable labeling vial (5.0 mL). The vial was sealed and degassed with N_2 for ∼10 min. The $^{99m}Tc(H_2O)_3(CO)_3^+$ precursor solution (100 μL) was added to the degassed vial and heated for 30 min at 80 °C. The reaction mixture was allowed to cool on an ice bath prior to analysis by radio-HPLC.

Method 2: Stepwise Formation. Picolinic acid (100 μL, 10^{-4} M) and phosphate buffer (700 μL, 0.1 M) at pH 7.4 were added to a sealable vial (5.0 mL). The vial was purged with nitrogen for ∼10 min. The $^{99m}Tc(H_2O)_3(CO)_3^+$ precursor solution (100 μL) was added to the degassed vial and heated for 30 min at 80 °C. After the formation of *fac*-$^{99m}Tc(H_2O)(CO)_3$(picolinate) was confirmed by radio-HPLC, ligand **3** (100 μL, 10^{-2} M) was added to the vial and the sample heated for an additional 30 min at 80 °C.

Biological pH Stability Studies. The respective ^{99m}Tc labeled compound (100 μL) prepared with ligands **2**, **3**, or **5** as directed above was added to phosphate buffer (900 μL, 0.1 M) at pH 7.4. The vial was sealed and incubated at 37.0 °C for the duration of the study. The solution was examined by radio-HPLC at 1, 2, and 4 h during the incubation to determine the effective stability of the compounds.

Mouse Serum Stability Studies. The respective ^{99m}Tc complexes (100 μL) prepared from ligands **2**, **3**, or **5** as directed above was added to an Eppendorf 1.5 mL flextube containing 400 μL of normal mouse serum with 0.1% sodium azide that had been previously clarified by centrifugation. The tube was sealed and constantly heated at 37.0 °C for the duration of the study. The serum was examined by radio-HPLC at 1, 2, and 4 h of incubation to observe decomposition or loss of the radiolabeled complex due to a shift in the retention time or decrease in the product peak intensity.

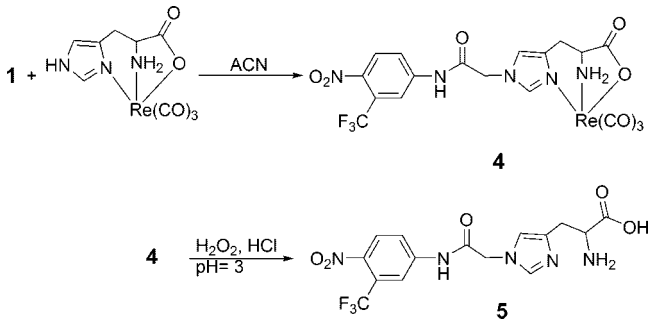
Cell Studies. *In vitro* analysis of the ^{99m}Tc complexes were conducted with DU-145 according to established methods prepared from ligand **2** or **5** as directed above was added. Aliquots of compounds (4, 6, 8 μL of a 0.1 mCi/100 μL) were incubated in triplicate with androgen receptor positive DU-145 prostate cancer cell line for 2 h in a CO_2 incubator at 37 °C. The cells were washed twice with cold PBS to remove nonspecific bound ^{99m}Tc complex; the cells were collected and counted with a Cobra II γ counter for 1 min.

RESULTS AND DISCUSSION

Nonsteroidal antiandrogen drugs, i.e., flutamide, bicalutamide, nilutamide, have been found to effectively mitigate early-stage prostate cancer growth (Figure 1) (*10*). We report within this paper the first analogues of flutamide that incorporate technetium-99m complexes into the structure. Flutamide was modified to incorporate a number of ligand systems capable of coordinating the ^{99m}Tc species, *fac*-$^{99m}Tc(OH_2)_3(CO)_3^+$ (Figure 2). We report within the synthetic methods used to prepare the bifunctional flutamide compounds, characterization of rhenium complexes formed, and radiochemical preparation and stability studies of the technetium-99m complexes formed.

The focus of this work was to develop a flutamide analogue that could be conveniently functionalized with a variety of ligand systems quite readily. The designs of such analogues were restricted to modifications along the carbonyl axis of flutamide's amide bond as the dominent binding motif for the compound to the androgen receptor is understood to be the functionalized aromatic ring (*6, 7, 10, 34, 35*). Transforming the alkyl backbone into an alkyl halide provided an accessible group that could function as a synthetic handle for nucleophilic substitution by a number of reactive species (i.e., amines, thiols, alcohols) (*13,15,36,37*). The brominated flutamide compound, 2-bromo-*N*-[4-nitro-3-(trifluoromethyl)phenyl]-acetamide, **1**, was chosen as the congener of fluatamide in order for later incorporation of chelators. Compound **1** was prepared in high yield by the reaction of bromoacetyl bromide and 5-amino-2-nitrobenzotrifluroride in CH_2Cl_2 in the presence of triethylamine (Scheme 1). The crude product **1** isolated from the reaction mixture after acid washing the reaction mixture was clean enough to use for the subsequent experiments without further purification based on ^{1}H and ^{13}C NMR spectroscopy. Compound **1** could also be further purified by silica gel chromatography (ethyl acetate/hexane 1:5) or

Scheme 2. Preparation of Histidine-Linked Flutamide Ligand (5) by Alkylation with 1 of the Rhenium Histidine to Yield the Rhenium Histidine-Linked Flutamide Complex (4) and Followed by Demetallation to Generate the Free Ligand (5)



recrystallized from ethyl acetate. Compound **1** was found to be a strong irritant as a dry powder, and care was utilized when handling the material.

The reactivity of the acetyl bromide in compound **1** provided an excellent synthetic handle to functionalize with corresponding ligand systems. Introduction of compound **1** afforded a series of molecules **2**, **3**, and **5** that incorporate flutamide and ligands (cysteine, imidazole, histidine, respectively). These ligands had been previously identified for coordination with a $M(CO)_3$ (M = Re, ^{99m}Tc) species due to high-efficiency labeling methods (*27, 28, 38*). The synthetic routes to **2**, **3**, and **5** are divided into two categories, direct organic synthesis and metal-coordinated synthesis. Direct synthesis involved a standard alkylation synthesis route to couple **1** with the ligand. The ligands cysteine and imidizole were attached to **1** via the direct synthesis route in a one-step substitution reaction to yield the flutamide-coupled ligands, compounds **2** and **3**, respectively (Scheme 1). The metal-coordinated synthesis of compound **5** involves the use of a rhenium as a protecting group on the histidine (Scheme 2).

Direct synthetic methods allowed the preparation of the compounds directly from compound **1** (Scheme 1). The cysteine-linked flutamide analogue, **2**, was successfully prepared in high yield after a number of synthetic attempts. The reported method has an important synthetic advantage by utilizing the reactive differences between thiol and amine groups to yield the desired product without the use of protecting groups. Early attempts utilizing protecting groups on cysteine on the amine and the carboxylic acid with Boc-Cys-OMe did yield the corresponding alkylation product with **1**. However, deprotection with base of the methyl ester was found to also cleave the amide bond as indicated by the presence of 5-amino-2-nitrobenzotrifluroride in the reaction mixture. Potential amide cleavage was anticipated at high pH due to the strong electron-withdrawing groups (NO_2, CF_3) on the aromatic ring weakening the amide bond. By treatment of **1** with excess cysteine in aqueous conditions at slightly basic pH, alkylation selectivity of the thiol over the amine in cysteine and the avoidance of protecting groups that require basic deprotection methods yielded the desired product, **2**. Initially, the reaction was conducted in aqueous solution only, but it proceeded slowly with low yields (<25%) due to the limited solubility of compound **1**. Attempts to improve reactivity and solubility with increased base and/or heat yielded undesired cleavage products. It is noteworthy to mention that, when the reaction was carried out in biphasic reaction conditions (aqueous/methylene chloride), compound **1** readily dissolved in the methylene chloride and the reaction proceed smoothly without the need of a phase transfer catalyst at room temperature with a significant improvement in yield. Careful control of the solution pH in the reaction was critical to yield the desired product, **2**. The cystine dimer was observed as the major product instead of **2** when the reaction was carried out in acidic conditions (pH < 5). Other water-miscible solvents (i.e., DMF, methanol) did dissolve **1**; however, the reaction did not afford compound **2** in any appreciable yield. The conditions reported here are mild for alkylation of a thiol compared to refluxing 1 M NaOH that can lead to racemic mixtures or utilizing carcinogenic solvents. Ligand **2** was characterized by elemental analysis and ^{1}H and ^{13}C NMR spectroscopy.

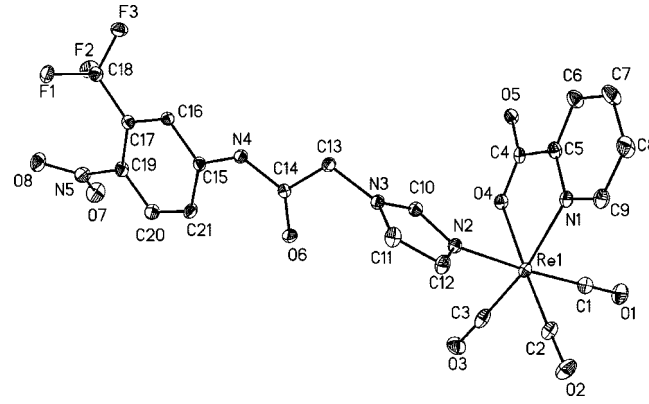
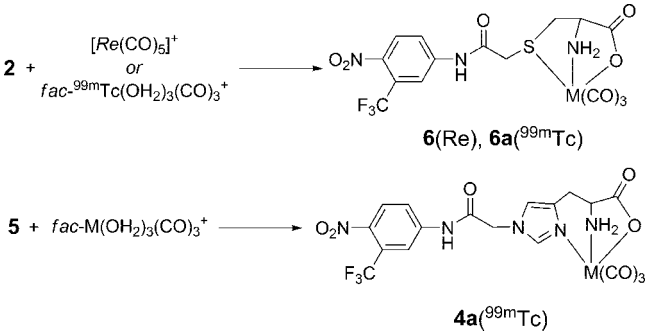


Figure 3. A modeled X-ray structure of *fac*-$Re(CO)_3$(Picolinate)(**3**), **7**, with 30% thermal ellipsoids. Hydrogen atoms are excluded for clarity.

An imidazole-linked flutamide analogue was prepared by treatment of **1** with excess imidazole in acetontrile gave **3** in high yield (Scheme 1). Under the reported reaction conditions, only the monosubstituted product was isolated, where the imidazole ligand was functioning as both the reactant and the proton scavenger. However, when the imidazole was added in stoichiometric concentrations and in the presence of an additional base (i.e., Cs_2CO_3) in acetonitrile, a mixture of the mono- and disubstituted products were observed. The mixed species were also observed when the reaction was conducted in a biphasic solvent system ($H_2O/MeCl_2$) similar to compound **2**. ^{1}H and ^{13}C NMR reported for **3** were consistent with the proposed compound.

Preparation of a N^{ϵ} histidine-linked flutamide compound ligand with **1** was originally attempted utilizing protecting groups similarly to previous reports (*28*, *39*, *40*). However, deprotection methods yielded cleavage products at the amide bond as noted previously in the synthesis of **2**. Therefore, utilizing a metal-coordinated synthetic route for the preparation of the ligand was a more appropriate method. In this procedure, $Re(CO)_3$ was coordinated in a tridentate fashion to the histidine ligand in place of more traditional organic protecting group (*28*). Alkylation of $Re(CO)_3$(histidine) with compound **1** in the presence of solid Cs_2CO_3 in an acetonitrile solution yielded compound **4** in near quantitative yields (Scheme 2). The reaction proceeds smoothly with no unwanted side products as indicated by HPLC, as the metal coordination bonds sufficiently protect the bound amine, carboxylate, and imidizole functional groups from substitution reactions (Figure 3). The coordinated imidazolyl ligand was of particular importance to maintain the coordination mode and strength for the subsequent ^{99m}Tc labeling. Dissociation of the nitrogen ligands from the rhenium center could lead to substitution at the NH_2 terminus or N^{τ} or disubstitution of the ring, similar to what was observed as a side product in the formation of compound **3**. The complexes were characterized by NMR, and comparison of **4** to the $Re(CO)_3$(Histidine) starting material illustrated similar splitting and slight downfield shifts for the protons associated with the coordinated histidine, indicating no change in the coordination mode of the ligand on the metal center. ^{1}H NMR of **4** in acetone showed two peaks associated with the imidazolyl group at 8.26

Scheme 3. Complexation of Flutamide-Linked Ligand Analogues with *fac*-M(CO)$_3$ (M = Re, ^{99m}Tc (a)) with Tridentate Ligands 2 and 5 to Generate *fac*-M(CO)$_3$(2) (6, 6a) and *fac*-M(CO)$_3$(5) (4, 4a) Complexes



and 7.18 ppm, slightly shifted downfield from the Re(CO)$_3$(Histidine) starting material at 7.06 and 8.08 ppm. The aromatic protons of the molecule were overlapped in the spectrum to yield a multiplet at 8.12 ppm of 3H. The metal coordinated amine protons are observed as diastereotopic protons of equal integration of one proton as a doublet at 5.4 and mulitplet at 5.75 ppm. The chiral Hα was also observed as a multiplet at 4.35 ppm, and the beta protons Hβ were also diastereotopic, exhibiting a dd pattern at 3.51 and 3.37 ppm.

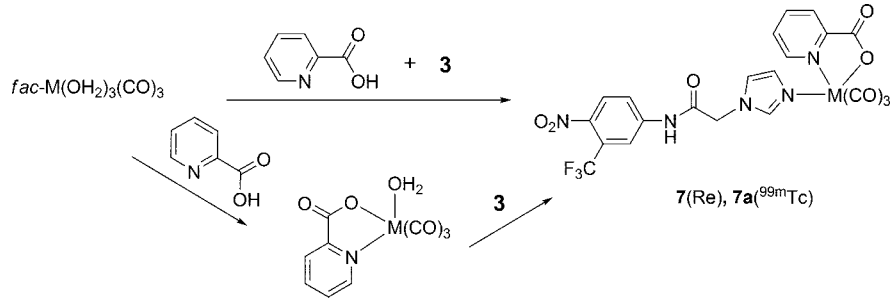
Removal of the coordinated rhenium tricarbonyl complex in **4** to generate the free ligand compound **5** was achieved under acidic oxidative conditions (Scheme 2). The selective oxidation and decomplexation of the rhenium center of **4** occurs by the addition of a 10-fold quantity of H_2O_2 under acidic conditions at room temperature. The reaction was monitored by HPLC and was completed after 3 days. Although a lower pH and increased H_2O_2 concentration would potentially decrease the reaction time, minimal amide cleavage products of the ligand were observed under the conditions reported. The free ligand **5** was isolated by preparatory HPLC from the reaction mixture in reasonable yields. Characterization of **5** by ^{1}H and ^{13}C NMR was consistent with the proposed structure and simplified in comparison to the corresponding rhenium complex, **4**.

Complexes were prepared from the ligands prepared above with rhenium and technetium-99m carbonyl precursors to yield M(CO)$_3$(Ligand/s) complexes. The preparation and characterization of the rhenium complexes provides a stable compound for characterization by standard methods and a reference for comparison to the radioactive ^{99m}Tc complexes in HPLC (Figure 2). The rhenium complexes of the flutamide-linked ligands were prepared by reacting the ligand with a rhenium carbonyl starting material as indicated in Scheme 3, except for complex **4a**, which was prepared directly from the Re(CO)$_3$(Histidine) complex as mentioned above. Ligands **2** and **3** utilized slightly different Re starting materials according to the solubility of the ligand system. Ligand **2** was reacted with the organic soluble [Re(CO)$_5$OTf] in methanol, due to the limited solubility of macroscopic amounts of **2** in water. The reaction involves the displacement of two CO molecules from the rhenium center upon complexation of **2** to generate the neutral complex Re(CO)$_3$(**2**), **6**, in high yield and isolated as a pale yellow solid. HPLC analysis of the product showed a single sharp peak with retention time of 22.1 min (Figure 2). Elemental analysis of **6** confirmed the existence of 1:1 ratio of rhenium to ligand. ^{13}C NMR profile of **6** is in consistent with the proposed structure. Relatively broad signals were observed in the ^{1}H NMR spectrum of **6** in CD$_3$OD at room temperature. At low temperature (−14 °C), sharp signals were observed, showing two sets of signals indicative of two coordination isomers in solution. The coordinated ligand **2** has a chiral carbon and prochiral sulfur resulting in the formation of diastereoisomers upon metal coordination. Two isomers can equilibrate fast by inversion of the sulfur configuration at room temperature. However, the minor species was observed at approximately 10% in the solution, indicating a preference for one coordination species with the Re(CO)$_3$ most likely due to interactions of functionalized thioether moiety driven by steric factors. Similar observations were reported for other thioether derivatives of cysteine Re(CO)$_3$ complexes (*26*, *38*).

Mundwiler et al. (*41*) reported a "2 + 1" mixed-ligand complex approach consisting of a bidentate and a monodentate ligand coordinated to the M(CO)$_3$ (M = Re, ^{99m}Tc) core provides an alternative method to single ligand coordination. This methodology was adopted to prepare a "2 + 1" flutamide derivative with the M(CO)$_3$ M = Re, ^{99m}Tc core. The rhenium complex, Re(CO)$_3$(pic)(**3**), **7**, prepared within this paper consisted of a bidentate picolinate and a monodentate flutamide fuctionalized imidazole, **3**. Two synthetic approaches, one-pot and stepwise synthesis, were attempted to prepare complex **7** (Scheme 4). The one-pot reaction yielded some of the product **7**; however, several other species were observed in the reaction mixture attributing multiple coordinating ligands of **3**. The stepwise synthesis provided a more clearly defined product in high yields. The compound *fac*-Re(OH$_2$)(CO)$_3$(picolinate) was prepared and isolated according to previous methods (*27*) and reacted with **3** in methanol to yield compound **7** as the only product in the HPLC chromatogram (Figure 2). Elemental analysis, ^{1}H and ^{13}C NMR as well as X-ray crystallography confirm the formation of complex **7**. ^{1}H NMR spectrum of **7** had increased overlapping signals at ∼8 ppm of the aromatic protons from the four protons of the coordinated picolinate and the three protons **3**. The methylene protons of **3** displayed a singlet signal, in contrast to doublet signals observed in **4**, suggesting that the monodentate ligand **3** is more flexible than the analogous tridentate ligand **5** in rhenium complexes. Crystals suitable for X-ray crystallography were obtained by diffusion of hexane into acetone solution of **7** (Figure 3). The structural parameters are listed in Table 1 and selected bond angles (°) and distances (Å) in Table 2. The rhenium center in complex **7** is a distorted octahedron with three CO ligands occupying the facial geometric sites in near-equivalent Re−C distances (1.89−1.92 Å) and C−Re−C angles (88.4−90.2°) with respect to each other. The other coordination sites are occupied by the coordinated "2 + 1" ligands, picolinate (pic) and **3**. Pic functions as a planar bidentate ligand (N, O) with distances and angles comparable to other reported structures (*27*, *41*, *42*). The monodentate ligand **3** is coordinated to the rhenium center through an imidazole nitrogen at N(2)−Re(1) 2.186 Å. The imidazole ligand itself is slightly tilted toward the plane of the pic ligand (∼83°) yielding an acutely distorted octahedron along the pic and **3** coordination sites. Compound **7** also exhibits interesting intermolecular NH−O (2.79 Å) hydrogen bonding between two molecules through the amide proton donor and the noncoordinated oxygen of the picolinate as a proton acceptor, yielding two hydrogen bond interactions per set of molecules in the unit cell.

Investigation of the radioactive ^{99m}Tc complexes were conducted by reacting ligands (**2**, **3**, and **5**) with the *fac*-[^{99m}Tc(CO)$_3$(H$_2$O)$_3$]$^+$ generated from a Tyco Isolink kit in aqueous conditions. The general labeling procedure for tridentate ligands **2** and **5** involved mixing the ligand with the *fac*-[^{99m}Tc(CO)$_3$(H$_2$O)$_3$]$^+$ at pH 7.4 and heating at 70 °C for 30 min (Scheme 3). Concentrations (10^{-4}, 10^{-5}, 10^{-6} M) were investigated at these conditions to determine effective labeling of the ligand. Both ligands **2** and **5** demonstrated good labeling of >90% at 10^{-4} and 10^{-5} M to yield *fac*-[^{99m}Tc(CO)$_3$(**2**)] **4a** and *fac*-[^{99m}Tc(CO)$_3$(**5**)] **6a** complexes (Figure 4). Labeling yields for both ligands **2** and **5** at 10^{-6} M were slightly diminished, 28% and 20% respectively, compared to the

Scheme 4. 2 + 1 Labeling Strategy for Reacting Ligand 3 with *fac*-$M(OH_2)_3(CO)_3$ (M = Re, ^{99m}Tc (a)) to Yield Complexes *fac*-$M(CO)_3$(pic)(3), (7, 7a)[a]



[a] Two strategies are reported: (1) single pot reaction; (2) stepwise addition of ligands.

Table 2. Selective Bond Angles (°) and Distance (Å) of *fac*-Re(CO)$_3$(Picolinate)(3), 7

bond lengths [Å]		angles [°]	
C(1)−Re(1)	1.920(3)	C(2)−Re(1)−C(3)	89.79(11)
C(2)−Re(1)	1.898(3)	C(2)−Re(1)−C(1)	88.41(11)
C(3)−Re(1)	1.902(3)	C(3)−Re(1)−C(1)	90.24(10)
N(1)−Re(1)	2.185(2)	C(2)−Re(1)−O(4)	175.05(9)
N(2)−Re(1)	2.186(2)	C(3)−Re(1)−O(4)	94.71(9)
O(4)−Re(1)	2.1424(17)	C(1)−Re(1)−O(4)	93.60(9)
		C(2)−Re(1)−N(1)	100.09(10)
		C(3)−Re(1)−N(1)	169.25(9)
		C(1)−Re(1)−N(1)	94.20(9)
		O(4)−Re(1)−N(1)	75.26(7)
		C(2)−Re(1)−N(2)	93.93(9)
		C(3)−Re(1)−N(2)	92.73(9)
		C(1)−Re(1)−N(2)	176.23(9)
		O(4)−Re(1)−N(2)	83.84(7)
		N(1)−Re(1)−N(2)	82.48(7)

Table 3. *fac*-^{99m}Tc$(OH_2)_3(CO)_3^+$ Labeling Yields Prepared at 30 min at 70 °C with pH 7.4 at Various Concentrations with Ligands (2, 3, and 5)

ligand (M)	10^{-4}	10^{-5}	10^{-6}
^{99m}Tc(CO)$_3$(**5**), (**4a**)	>99%	95%	20%
^{99m}Tc(CO)$_3$(**2**), (**6a**)	>99%	90%	28%
		10^{-2}	
Single Pot[a]			
^{99m}Tc(CO)$_3$(pic)(**3**), (**7a**)		71	
Stepwise Addition[b]			
^{99m}Tc(CO)$_3$(pic)(**3**), (**7a**)		70	

[a] The picolinate (pic) at 10^{-5} M and **3** were added to directly to the same vial. [b] ^{99m}Tc(CO)$_3$ was added to a picolinate (10^{-5} M) solution and heated for 30 min, then followed by the addition of **3** and 30 min additional heating.

Table 4. Stability Studies of ^{99m}Tc Complexes (4a, 6a, and 7a) Formed with Ligands (2, 3, and 5) at Biological pH (7.4) at 37 °C and in Normal Mouse Serum at 37 °C

	1 h	2 h	4 h
pH = 7.4 at 37 °C			
^{99m}Tc(CO)$_3$(**5**), (**4a**)	>95	>95	>95
^{99m}Tc(CO)$_3$(**2**), (**6a**)	88	88	88
Normal Mouse Serum at 37 °C			
^{99m}Tc(CO)$_3$(**5**), (**4a**)	85	82	80
^{99m}Tc(CO)$_3$(**2**), (**6a**)	95	95	95
^{99m}Tc(CO)$_3$(pic)(**3**), (**7a**)	81	77	70

previously reported ^{99m}Tc labeling with similar ligands at higher temperatures (95 °C) (*28*, *38*, *43*). When the reaction of *fac*-$[^{99m}Tc(CO)_3(H_2O)_3]^+$ with **2** or **5** was carried out at 95 °C, decreased labeling yields of **4a** and **6a** and the corresponding amide cleavage product as a ^{99m}Tc(CO)$_3$ free carboxylate ligand complex were observed in the radiochromatograms. The pH of

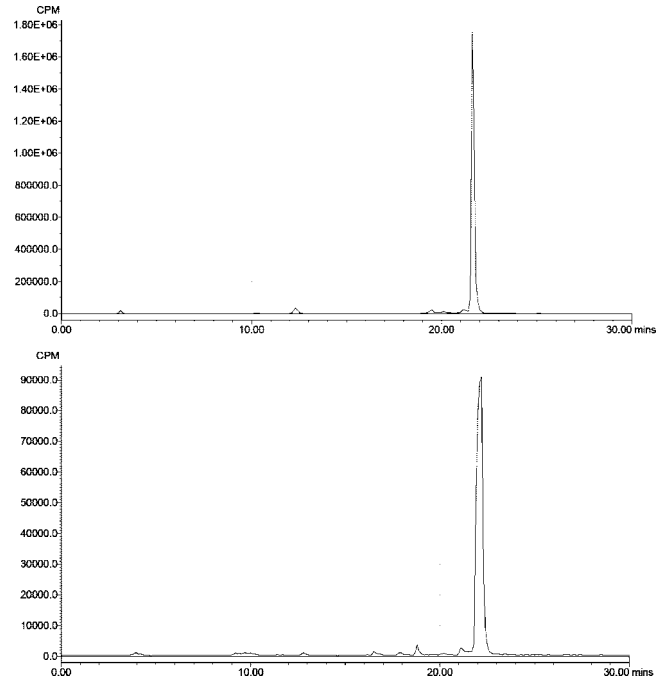


Figure 4. Radio HPLC of the ^{99m}Tc(CO)$_3$ complexes **4a** (top) and **6a** (bottom) formed with ligands **5** and **3**, respectively.

the labeling solution was also found to significantly impact the labeling yields observed. Reactions conducted in slightly acidic conditions between pH 6.5 and 7.4 would yield the desired product, **4a** and **6a**, without the appearance of cleavage products. Attempts to prepare **4a** and **6a** by direct addition of Isolink kit to the reaction mixture in 0.1 M phosphate buffer partially yielded the products and cleavage products. However, neutralization of the Isolink kit with hydrochloric acid to pH ~7.0 prior to addition to the ligand solution was found to be the imperative step in the labeling procedure as the kit solution is quite basic (pH ~11) during the production of *fac*-$[^{99m}Tc(CO)_3(H_2O)_3]^+$. Despite the combination of heating and pH effects impacting the labeling yields, carefully control of the reaction conditions afforded the desired compounds in good yield. The complexes **4a** and **6a** were further studied to determine the stability of the compound in solution at 37 °C in phosphate buffer (pH 7.4) and in mouse serum at 1, 2, and 4 h (Table 4). Both compounds, **4a** and **6a**, were found to be stable for up to four hours in the pH stability and the serum studies with minimal decomposition of complex or cleavage products observed in the radio-HPLC.

The "2 + 1" ^{99m}Tc complex, **7a**, with ligand **3** was prepared by similar methods to the rhenium analogues: one-pot and stepwise (Scheme 4). The preparation of **7a** by either method at 70 °C and pH 7.4 yielded a peak with a similar retention

time to the analogous rhenium complex (Table 3). Each of these methods had comparable labeling results of ~70%. The formation of the "2 + 1" complex **7a** required much higher concentrations (10^{-2} M) of **3** compared to the tridentate ligands **2** and **5**. HPLC purified complex **7a** was incubated in phosphate buffer (0.1 M, pH 7.4) and 37 °C to determine stability of the complex at 1, 2, and 4 h (Table 4). During this study, ligand **3** was observed to partially dissociate during incubation from complex **7a** to yield the intermediate species *fac*-$^{99m}Tc(CO)_3(H_2O)$(pic) as identified by retention time. Mouse serum studies further confirmed the instability of the "2 + 1" complex as multiple species in addition to the dissociation of **3** were observed in the radio-HPLC. Despite the previously reported and successful synthesis with the rhenium analogue (*41*), the "2 + 1" complex **7a** containing functionalized imidazole **3** may have limited application due to stability of the complex.

In vitro analysis were conducted with the ^{99m}Tc complexes (**4a**, **6a**) to determine cellular uptake with an androgen-positive DU-145 prostate cancer cell line. Incubation of the ^{99m}Tc complexes with the prostate cancer cells displayed a modest ~2% binding relative to control experiments. Although the uptake was much lower than expected, the decrease binding of these particular model compounds was anticipated. The changes in the chemical nature (i.e., polarity, steric bulk) of the native flutamide compound by introduction of the ^{99m}Tc complexes may have impacted the internalization of the modified compounds. Additionally, the functionalized flutamide complexes may also have decreased AR affinity due to poor metabolic conversion. The native flutamide compound is metabolized to the more potent α-hydroxyl antagonist, hydroxyflutamide, by the AR. Although it is uncertain the potential affect of the ^{99m}Tc complexes will have on the AR with limited internalization of the compounds, additional studies will be required.

CONCLUSION

The study presented here demonstrates the first $M(CO)_3$-linked flutamide compounds (M = Re, ^{99m}Tc) prepared for prostate cancer imaging. Although the amide bond of the flutamide was considered to be unstable due to hydrolytic cleavage, conditions were found to prepare the compounds in high yield. Reactions involving *fac*-$^{99m}Tc(CO)_3(H_2O)_3$ with tridentate ligands, cysteine and histidine, offered higher labeling yields and greater stability than the "2 + 1" approach utilizing a functionalized imidazole analogue. The tridentate ligands exhibited excellent radiochemical stability at 37 °C at physiological pH and in mouse serum. *In vitro* assays did show limited uptake in DU-145 prostate cancer cells. However, further investigations are required to conclusively determine internalization mechanism and androgen inhibition. Further studies involving metabolic activation of $^{99m}Tc(CO)_3$ linked flutamide complexes to the hydroxyflutamide analogue and *in vivo* tumor model experiments are currently underway.

ACKNOWLEDGMENT

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Supporting Information Available: X-ray structural information for **7** (cif). This material is available free of charge via the Internet at http://pubs.acs.org.

LITERATURE CITED

(1) Jemal, A., Siegel, R., Ward, E., Murray, T., Xu, J. Q., and Thun, M. J. (2007) Cancer statistics, 2007. *Ca-a Cancer Journal for Clinicians 57*, 43–66.

(2) http://www.cancer.gov/cancertopics/pdq/treatment/prostate/patient.

(3) http://www.prostate-cancer.com/.

(4) Culig, Z. R., Klocker, H., Bartsch, G., Steiner, H., and Hobisch, A. (2003) Androgen receptors in prostate cancer. *J. Urol. 170*, 1363–1369.

(5) DeMarzo, A. M., Nelson, W. G., Isaacs, W. B., and Epstein, J. I. (2003) Pathological and molecular aspects of prostate cancer. *Lancet 361*, 955–964.

(6) Singh, S. M., Gauthier, S., and Labrie, F. (2000) Androgen receptor antagonists (antiandrogens): Structure-activity relationships. *Curr. Med. Chem. 7*, 211–247.

(7) Gao, W. Q., Kearbey, J. D., Nair, V. A., Chung, K. W., Parlow, A. F., Miller, D. D., and Dalton, J. T. (2004) Comparison of the pharmacological effects of a novel selective androgen receptor modulator, the 5 alpha-reductase inhibitor finasteride, and the antiandrogen hydroxyflutamide in intact rats: New approach for benign prostate hyperplasia. *Endocrinology 145*, 5420–5428.

(8) Urushibara, M., Ishioka, J., Hyochi, N., Kihara, K., Hara, S., Singh, P., Isaacs, J. T., and Kageyama, Y. (2007) Effects of steroidal and non-steroidal antiandrogens on wild-type and mutant androgen receptors. *Prostate 67*, 799–807.

(9) Yin, D. H., He, Y. L., Perera, M. A., Hong, S. S., Marhefka, C., Stourman, N., Krikovsky, L., Miller, D. D., and Dalton, J. T. (2003) Key structural features of nonsteroidal ligands for binding and activation of the androgen receptor. *Mol. Pharmacol. 63*, 211–223.

(10) Bohl, C. E., Chang, C., Mohler, M. L., Chen, J. Y., Miller, D. D., Swaan, P. W., and Dalton, J. T. (2004) A ligand-based approach to identify quantitative structure-activity relationships for the androgen receptor. *J. Med. Chem. 47*, 3765–3776.

(11) Miller, D. D., He, Y. L., Marhefka, C. A., Rakov, I. M., Hong, S. S., Hwang, D. J., Mohler, M. L., Kirichenko, S. N., Mustafa, S. M., Nair, V. A., Patil, R., Chung, K. W., Veverka, K. A., Steiner, M. S., Barnette, K. G., Yin, D. H., Gao, W. Q., Kearbey, J. D., Xu, H. P., Kim, J., Chen, J. Y., Bohl, C., and Dalton, J. T. (2003) Novel selective androgen receptor modulators (SARMs). *Abstr. Pap. Am. Chem. Soc. 226*, U1-U1.

(12) Isaacs, J. T., and Isaacs, W. B. (2004) Androgen receptor outwits prostate cancer drugs. *Nat. Med. 10*, 26–27.

(13) Parent, E. E., Dence, C. S., Jenks, C., Sharp, T. L., Welch, M. J., and Katzenellenbogen, J. A. (2007) Synthesis and biological evaluation of [F-18]bicalutamide, 4-[Br-76]bromobicalutamide, and 4-[Br-76]bromo-thiobicalutamide as nonsteroidal androgens for prostate cancer imaging. *J. Med. Chem. 50*, 1028–1040.

(14) Payen, O., Top, S., Vessieres, A., Brule, E., Plamont, M. A., McGlinchey, M. J., Muller-Bunz, H., and Jaouen, G. (2008) Synthesis and structure-activity relationships of the first ferrocenyl-aryl-hydantoin derivatives of the nonsteroidal antiandrogen nilutamide. *J. Med. Chem. 51*, 1791–1799.

(15) Nair, V. A., Mustafa, S. M., Mohler, M. L., Yang, J., Kirkovsky, L. I., Dalton, J. T., and Miller, D. D. (2005) Synthesis of irreversibly binding bicalutamide analogs for imaging studies. *Tetrahedron Lett. 46*, 4821–4823.

(16) Nair, V. A., Mustafa, S. M., Mohler, M. L., Fisher, S. J., Dalton, J. T., and Miller, D. D. (2004) Synthesis of novel iodo derived bicalutamide analogs. *Tetrahedron Lett. 45*, 9475–9477.

(17) Choe, Y. S., Lidstrom, P. J., Chi, D. Y., Bonasera, T. A., Welch, M. J., and Katzenellenbogen, J. A. (1995) Synthesis of 11-*β*-[F-18]fluoro-5-α-dihydrotestosterone and 11-*β*-[F-18]fluoro-19-nor-5-α-Dihydrotestosterone - preparation via halofluorination-reduction, receptor-binding, and tissue distribution. *J. Med. Chem. 38*, 816–825.

(18) Jurisson, S. S., and Lydon, J. D. (1999) Potential technetium small molecule radiopharmaceuticals. *Chem. Rev. 99*, 2205–2218.

(19) Das, T., Banerjee, S., Samuel, G., Bapat, K., Subramanian, S., Pillai, M. R. A., and Venkatesh, M. (2006) A novel ^{99m}Tc-labeled testosterone derivative as a potential agent for targeting androgen receptors. *Bioorg. Med. Chem. Lett. 16*, 5788–5792.

(20) Aebischer, N., Schibli, R., Alberto, R., and Merbach, A. E. (2000) Complete carbonylation of *fac*-$[Tc(H_2O)_3(CO)_3]^+$ under CO pressure in aqueous media: A single sample story. *Angew. Chem.-Int. Ed. 39*, 254.

(21) Alberto, R., Pak, J. K., van Staveren, D., Mundwiler, S., and Benny, P. (2004) Mono-, bi-, or tridentate ligands? The labeling of peptides with ^{99m}Tc-carbonyls. *Biopolymers 76*, 324–333.

(22) Alberto, R., Schibli, R., Egli, A., Schubiger, A. P., Abram, U., and Kaden, T. A. (1998) A novel organometallic aqua complex of technetium for the labeling of biomolecules: Synthesis of $[^{99m}Tc(OH_2)_3(CO)_3]^+$ from $[^{99m}TcO_4]^-$ in aqueous solution and its reaction with a bifunctional ligand. *J. Am. Chem. Soc. 120*, 7987–7988.

(23) Alberto, R., Schibli, R., Schubiger, A. P., Abram, U., Pietzsch, H. J., and Johannsen, B. (1999) First application of *fac*-$[^{99m}Tc(OH_2)_3(CO)_3]^+$ in bioorganometallic chemistry: Design, structure, and in vitro affinity of a 5-HT1A receptor ligand labeled with ^{99m}Tc. *J. Am. Chem. Soc. 121*, 6076–6077.

(24) Alberto, R., Schibli, R., Waibel, R., Abram, U., and Schubiger, A. P. (1999) Basic aqueous chemistry of $[M(OH_2)_3(CO)_3]^+$ (M = Re, Tc) directed towards radiopharmaceutical application. *Coord. Chem. Rev. 192*, 901–919.

(25) Alberto, R., Ortner, K., Wheatley, N., Schibli, R., and Schubiger, A. P. (2001) Synthesis and properties of boranocarbonate: A convenient in situ CO source for the aqueous preparation of $[^{99m}Tc(OH_2)_3(CO)_3]^+$. *J. Am. Chem. Soc. 123*, 3135–3136.

(26) He, H. Y., Lipowska, M., Xu, X. L., Taylor, A. T., Carlone, M., and Marzilli, L. G. (2005) $Re(CO)_3$ complexes synthesized via an improved preparation of aqueous *fac*-$[Re(CO)_3(H_2O)_3]^+$ as an aid in assessing ^{99m}Tc imaging agents. Structural characterization and solution behavior of complexes with thioether-bearing amino acids as tridentate ligands. *Inorg. Chem. 44*, 5437–5446.

(27) Schibli, R., La Bella, R., Alberto, R., Garcia-Garayoa, E., Ortner, K., Abram, U., and Schubiger, P. A. (2000) Influence of the denticity of ligand systems on the in vitro and in vivo behavior of ^{99m}Tc(I)-tricarbonyl complexes: A hint for the future functionalization of biomolecules. *Bioconjugate Chem. 11*, 345–351.

(28) Pak, J. K., Benny, P., Spingler, B., Ortner, K., and Alberto, R. (2003) N^{ε} functionalization of metal and organic protected L-histidine for a highly efficient, direct labeling of iomolecules with $[Tc(OH_2)_3(CO)_3]^+$. *Chem. Eur. J. 9*, 2053–2061.

(29) Hope, H. (1994) X-ray crystallography - a fast, first-resort analytical tool. *Prog. Inorg. Chem. 41*, 41–119.

(30) *SAINTPlus: v. 6.45a and v.7.23a, Data Reduction and Correction Program* (2003/2004) Bruker AXS, Madison, WI.

(31) *SADABS: v.2.01, v. 2004/1 an empirical absorption correction program* (2004) Bruker AXS Inc., Madison, WI.

(32) *SHELXTL: v. 6.14, Structure Determination Software Suite* (2004) Sheldrick, G. M.,Bruker AXS Inc., Madison, WI.

(33) Alves, S., Correia, J. D. G., Gano, L., Rold, T. L., Prasanphanich, A., Haubner, R., Rupprich, M., Alberto, R., Decristoforo, C., Santos, I., and Smith, C. J. (2007) In vitro and in vivo evaluation of a novel $^{99m}Tc(CO)_3$-pyrazolyl conjugate of cyclo-(Arg-Gly-Asp-D-Tyr-Lys). *Bioconjugate Chem. 18*, 530–537.

(34) Soderholm, A. A., Lehtovuori, P. T., and Nyronen, T. H. (2005) Three-dimensional structure-activity relationships of nonsteroidal ligands in complex with androgen receptor ligand-binding domain. *J. Med. Chem. 48*, 917–925.

(35) Van Dort, M. E., and Jung, Y. W. (2001) Synthesis and structure-activity studies of side-chain derivatized arylhydantoins for investigation as androgen receptor radioligands. *Bioorg. Med. Chem. Lett. 11*, 1045–1047.

(36) Jacobson, O., Bechor, Y., Icar, A., Novak, N., Birman, A., Marom, H., Fadeeva, L., Golan, E., Leibovitch, I., Gutman, M., Even-Sapir, E., Chisin, R., Gozin, M., and Mishani, E. (2005) Prostate cancer PET bioprobes: Synthesis of [F-18]-radiolabeled hydroxyflutamide derivatives. *Bioorg. Med. Chem. 13*, 6195–6205.

(37) Van Dort, M. E., and Jung, Y. W. (2004) Synthesis and structure-activity investigation of iodinated arylhydantoins and arylthiohydantoins for development as androgen receptor radioligands. *Bioorg. Med. Chem. Lett. 14*, 5285–5288.

(38) van Staveren, D. R., Benny, P. D., Waibel, R., Kurz, P., Pak, J. K., and Alberto, R. (2005) S-functionalized cysteine: Powerful ligands for the labelling of bioactive molecules with triaquatricarbonyltechnetium-99m(1+) $[^{99m}Tc(OH_2)_3(CO)_3]^+$. *Helv. Chim. Acta 88*, 447–460.

(39) Narayanan, S., Vangapandu, S., and Jain, R. (2001) Regiospecific synthesis of 2,3-disubstituted-L-histidines and histamines. *Bioorg. Med. Chem. Lett. 11*, 1133–1136.

(40) Kovalainen, J. T., Christiaans, J. A. M., Kotisaari, S., Laitinen, J. T., Mannisto, P. T., Tuomisto, L., and Gynther, J. (1999) Synthesis and in vitro pharmacology of a series of new chiral histamine H-3-receptor ligands: 2-(R and S)-amino-3-(1H-imidazol-4(5)-yl)propyl ether derivatives. *J. Med. Chem. 42*, 1193–1202.

(41) Mundwiler, S., Kundig, M., Ortner, K., and Alberto, R. (2004) A new [2 + 1] mixed ligand concept based on $[^{99(m)}Tc(OH_2)_3(CO)_3]^+$: a basic study. *Dalton Trans.* 1320–1328.

(42) Benny, P. D., Fugate, G. A., Morley, J. E., Twamley, B., and Trabue, S. (2008) Synthesis and characterization of 2,5-bis(benzylthio)-1,3,4-thiadiazole complexes with *fac*-$ReBr_3(CO)_3^{2-}$. *Inorg. Chim. Acta*. in press.

(43) van Staveren, D. R., Mundwiler, S., Hoffmanns, U., Pak, J. K., Spingler, B., Metzler-Nolte, N., and Alberto, R. (2004) Conjugation of a novel histidine derivative to biomolecules and labelling with $[^{99m}Tc(OH_2)_3(CO)_3]^+$. *Org. Biomol. Chem. 2*, 2593–2603.

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Synthesis and characterization of 2,5-bis(benzylthio)-1,3,4-thiadiazole complexes with *fac*-$ReBr_3(CO)_3^{2-}$

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ABSTRACT

Reactions of 2,5-bis(benzylthio)-1,3,4-thiadiazole (**1**) with a common organometallic rhenium starting material $[NEt_4]_2[fac\text{-}[Re(I)Br_3(CO)_3]$ yielded two distinct types of complexes. Both complexes coordinate only through the nitrogen of the thiadiazole ring. Reaction of **1** with the rhenium starting material alone yielded a bimetallic complex *fac*-(di-μ-bromo)(μ-2,5-bis(benzylthio)-1,3,4-thiadiazole-κN:κ′N)bis(tricarbonyl rhenium (I)) (**2**). The nitrogens of the thiadiazole ring of **1** each coordinate to a different rhenium center combined with two bridging bromide ligands in **2**. A "2+1" complex was prepared in a two step process by reacting the rhenium starting material with picolinic acid followed by **1** to yield *fac*-Re(I)(2,5-bis(benzylthio)-1,3,4-thiadiazole)$(CO)_3$(picolinate) (**3**). Compounds **2** and **3** were characterized by NMR, IR, UV, elemental analysis, and single crystal X-ray diffraction.

1. Introduction

1,3,4-Thiadiazole-2,5-dithiolate (TDD) and analogs have a broad range of potential applications (i.e., solid state organic crystals, pharmaceuticals, bioinorganic, catalysis) [1–15]. A number of different types of complexes with TDD have been observed with a wide spectrum of transition metals (Scheme 1). TDD can coordinate through a several different modes as a mondentate ligand through one of the sulfur donors or a nitrogen in the thiadiazole ring [2–6]. A number of bidentate complexes can also be formed with TDD where the ligand can interact with the metal center through two sulfur donors similar to a dithiocarbamates or through a combination of thiol and diazole nitrogen analogous to a thioacetimide. The TDD ligand can function as a bidentate ligand with a single metal center [4,7–9] or as a bridging ligand forming a bimetallic complex [8–15]. TDD can also be functionalized by conversion of the thiols into thioethers producing a number of derivatives that provide tunability of the ligand system (Scheme 1). Additional donor ligands (i.e., pyridine, carboxylic acid, acetylacetone) modify the coordination number of the ligand, donors effect, complexes isolated and the chemistry of the complex [11,12,16,7,17].

TDD rhenium complexes have particular applications in photochemical materials, nonlinear optics and radiopharmaceutical applications as a surrogate for technetium-99m complexes used in nuclear medicine imaging. Generally, the isolated TDD complexes have been primarily isolated with the rhenium center in lower oxidation states (+1 to +3). Rhenium and technetium(III) complexes formed by reacting a benzothiazole-2-thiol analog revealed a bidentate (S, N) coordination mode with the metal centers [18]. Recently, organometallic rhenium analogs have been reacted with TDD. A common rhenium starting material, $Re(CO)_5(SO_3CF_3)$, was reacted with TDD to yield a mixed monodentate coordination complex [19]. Three octahedral rhenium metal centers were coordinated to a single TDD ligand through the thiols and the nitrogen of the 1,3,4-thiadiazole ring and no displacement of CO ligands was observed. A simplified TDD analog, a diazole ligand was incorporated into tridentate ligand to coordinate to *fac*-$M(CO)_3(OH_2)_3^+$ M = Re, Tc, where a mixture of coordinated diazole species were identified in the reaction mixture [20].

The interesting electronic properties of TDD and the functional capability through alkylation of the thiol into a thioether containing ligand led to the investigation of the thioether analogs with rhenium. Alberto recently reported a benzyl thioether rhenium complex, *fac*-$Re(CO)_3$(*S*-benzyl cysteine), and the corresponding technetium complex, in which the sulfur in the thioether moiety was an acceptable donor for the organometallic core [21]. This paper investigates the interactions of 2,5-bis(benzylthio)-1,3,4-thiadiazole (**1**) with *fac*-$ReBr_3(CO)_3^{2-}$ focusing on the coordination chemistry modes of the ligand and complexes observed. The benzyl analog serves as an important model system for examining the fundamental interactions of the ligand with the *fac*-rhenium center prior to additional electronic tuning of the molecule and

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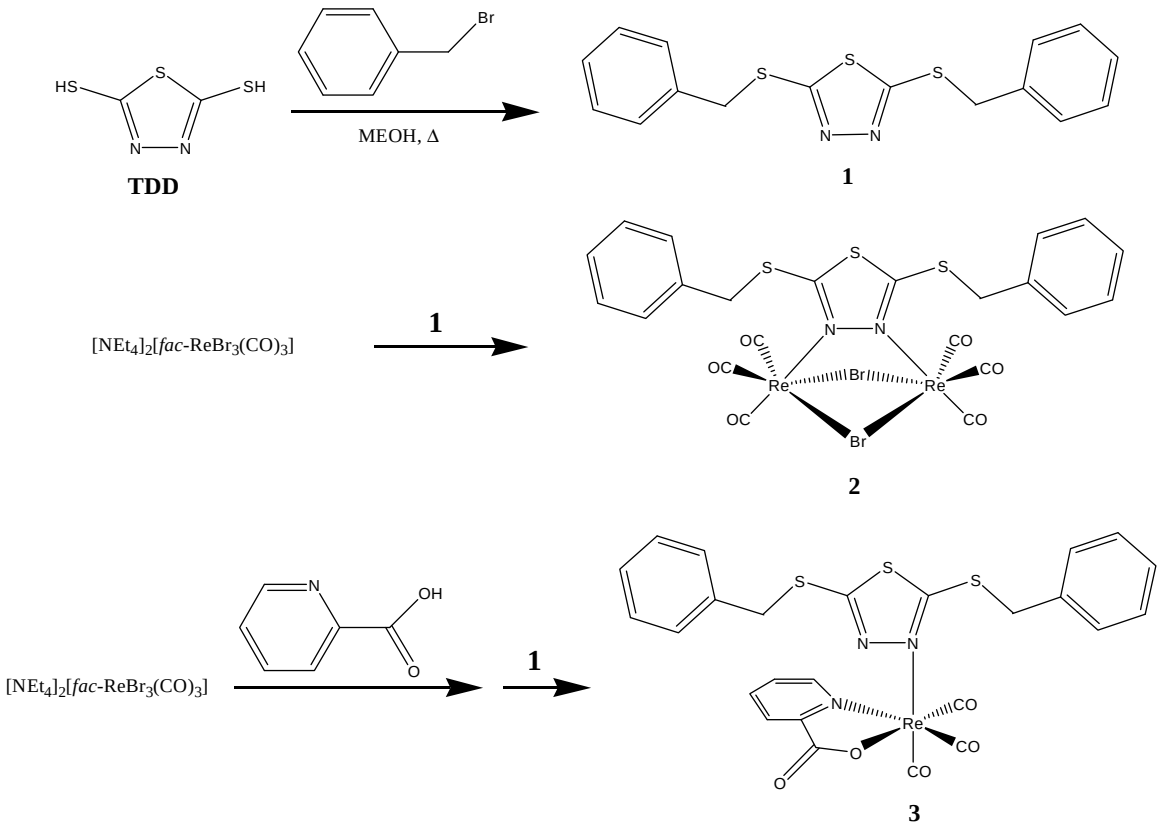

Scheme 1. Synthetic procedures for the formation of **1**, **2** and **3**.

the corresponding absorbance and emission capabilities of the complex.

2. Experimental

2.1. General details

All reagents were of ACS grade or higher were purchased from Aldrich, Fluka, Acros, Strem or Alfa Aesar and were used without further purification. $[NEt_4]_2[fac\text{-}[ReBr_3(CO)_3]$ was prepared in a two step procedure from dirhenium decacarbonyl according to literature methods [22]. ^1H and ^{13}C NMR spectra were recorded at 293 K on a Varian Mercury Vx 300 spectrometer using 5 mm NMR tubes and processed using Varian VNWR 6.1 software using trimethylsilane and/or solvent peaks as an internal reference. IR spectra were recorded on a Thermo Nicolett 6700 FT-IR equipped with an ATR cell and analyzed with OMNIC 7.1a software. UV/Vis spectra were recorded of the compounds in methanol in quartz cuvettes on a Varian Cary 50 Bio and analyzed with Cary WINUV 3.00 software. Elemental analyses were performed by QTI of Whithouse, NJ. Mass spectra of the samples were obtained by electrospray ionization directly infusing the samples into an Agilent 1100 Ion Trap LC/MS/MS and scanning from 50 to 1200 *m/z* with the drying gas at 12 mL min^{-1} at 350 °C and nebulizer pressure set at 50 psig.

Separation and identification of compounds were conducted on a Perkin Elmer Series 200 High Pressure Liquid Chromatograph (HPLC) with an Agilent Zorbex 30 cm SB-C18 column. The reverse phase gradient system begins with 0.1% trifluoroacetic acid (TFA) aqueous eluent gradually shifting to methanol according to the following method, 0–3.0 min (100% TFA), 3.0–9.0 min (75% TFA, 25% MeOH), 9.0–20.0 min (25–100% MeOH linear gradient), 20.0–25.0 min (100% MeOH) at a flow rate of 1.0 mL/min.

2.2. Preparation of compounds

2.2.1. 2,5-Bis(benzylthio)-1,3,4-thiadiazole (*1*)

2,5-Bis(benzylthio)-1,3,4-thiadiazole (**1**) was prepared by modifying a previously reported procedure to significantly improve yields and purity of the compound [1]. Compound **1** was prepared by combining ~~1,3,4-thiadiazole-2,5-dithiol~~ (2.0 g, 0.0133 mol) with benzylbromide (4.76 g, 0.0280 mol) and triethylamine (2.828 g, 0.0280 mol) in 50.0 mL methanol. The solution was stirred and refluxed for 2 h. Upon cooling, the formation of analytically pure white crystalline solid of **1** was observed. The solid was collected by filtration and washed with methanol to yield pure product. Yield 4.038 g, 92%. ^1H NMR (273 K, CD_3COCD_3) 4.54 (4H, s), 7.27–7.38 (3H, m), 7.44–7.48 (2H, m) ^{13}C NMR (273 K, CD_3COCD_3) 38.1, 128.0, 128.8, 129.4, 136.7, 164.8 UV/Vis (nm, (ε)) 294.0 (12, 590) HPLC RT 24.4 min.

2.2.2. [fac-$Re_2(CO)_6(\mu\text{-}Br)_2$(μ-5-bis(benzylthio)-1,3,4-thiadiazole)] (*2*)

Compound **1** (0.100 g, 0.301 mmol) was combined with *fac*-[$NEt_4]_2[Re(I)Br_3(CO)_3$] (0.211 g, 0.274 mmol) in 15 mL of methanol. The solution was stirred for 9 h at 60 °C under nitrogen. The methanol was removed by rotary evaporation at 40 °C followed by drying *in vacuo*. The solid was washed three times with methanol to remove residual **1** and $NEt_4^+Br^-$. The solid was redissolved in methylene chloride, filter and evaporated to yield the pure complex **2**. Single crystals suitable for X-ray diffraction were produced by slow infusion of pentane into a methylene chloride solution of the complex. Yield: 0.058 g, 41.4%. ^1H NMR (chloroform-d_3): δ = 7.42–7.62 (m, 10H, aromatic H), 4.37 (s, 4, CH_2). ^{13}C NMR (chloroform-d_3): δ = 41.0, 129.0, 129.5, 129.6, 129.7, 131.8, 174.3. IR (solid, cm^{-1}) 2045 m, 2019 m, 1937 s, 1909.1 s, 1889.7 s, 906 s, 729 s, 700 m. UV/Vis (nm, (ε)) 272.0 (45, 900). *Anal.* calc. for $C_{22}H_{14}Br_2N_2O_6Re_2S_3$.

0.75CH_3OH: C, 25.9; H, 1.62; N, 2.65. Found: C, 28.86; H, 1.64; N, 3.50%.

2.2.3. *[fac-Re(CO)$_3$(picolinate)(5-bis(benzylthio)-1,3,4-thiadiazole)]* (**3**)

Picolinic acid (0.035 g, 0.286 mmol) and $[NEt_4]_2$[*fac*-$Re(CO)_3Br_3$] (0.200 g, 0.260 mmol) were dissolved into 10.0 mL of methanol. Sodium bicarbonate (0.290 mmol) was added to adjust the pH to neutral. The stirred solution was heated at 70 °C under nitrogen for 1 h when HPLC indicated the formation of $Re(CO)_3(OH_2)$(picolinate) was complete (RT 18.5 min). Compound **1** (0.094 g, 0.286 mmol) was added to the solution and heated at 70 °C overnight after which time HPLC indicated the formation of *fac*-[$Re(CO)_3$(picolinate) (**1**)] (**3**; RT 23.5 min). Upon evaporation of the solution an off-white precipitate was collected and washed with water multiple times. The product, **3,** was isolated by silica chromatography (RF 0.15; 3:1 ethyl acetate:hexane). Single crystals suitable for X-ray diffraction were produced by slow evaporation of the complex solution in mixed media of acetonitrile and water. Yield: 0.098 g, 48.8%. ^{1}H NMR (chloroform-d_3): δ = 8.76 (d, 1H, HC), 8.13 (d, 1H, aromatic H), 7.92 (t, 1H, aromatic H), 7.40 (m, 6H, aromatic H), 7.29 (m, 3H, aromatic H), 7.14 (m, 2, aromatic H), 4.326 (s, 2, benzyl), 4.18 (d, $1H_a$, J_{ab} = 12.9, benzyl) 3.98 (d, $1H_b$, J_{ab} = 12.9, benzyl). ^{13}C NMR (chloroform-d_3): δ = 174.8, 173.0, 163.8, 152.0, 151.9, 139.4, 134.3, 133.1, 129.2, 129.2, 129.1, 128.9, 128.9, 128.8, 128.3, 128.0, 127.2, 126.4, 107.3, 41.2, 38.4. IR (solid, cm^{-1}) 2017 s, 1889 s, 1637 m, 1598 m, 1349 m, 774 m, 691 m. UV/Vis (nm, (ε)) 286.9 (17, 320). MS M^+ 745.9. *Anal.* calc. for $C_{25}H_{18}N_3O_5ReS_3$: C, 41.54; H, 2.51; N, 5.81. Found: C, 41.83; H, 2.80; N, 5.89%.

2.3. X-ray experimental

Crystals of **2** and **3** were removed from the flask and covered with a layer of hydrocarbon oil. A suitable crystal was selected, attached to a glass fiber and placed in the low-temperature nitrogen stream [23]. Data for **2** and **3** were collected at ca. 89 K using a Bruker/Siemens SMART APEX instrument (Mo Kα radiation, λ = 0.71073 Å) equipped with a Cryocool NeverIce low temperature device. Data were measured using omega scans of 0.3° per frame for 5 s for both. A full sphere of data was collected in each case with a total of 2400 frames and a final resolution of 0.83 Å. Cell parameters were retrieved using SMART [24] software and refined using SAINTPLUS [25] on all observed reflections. Data reduction and correction for Lp and decay were performed using the SAINTPLUS software. Absorption corrections were applied using SADABS [26]. Each structure was solved by direct methods and refined by least squares method on F^2 using the SHELXTL program package [27]. Compound **2** was solved in the space group $P2(1)/n$ (#14) and **3** in $P\bar{1}$ (#2) by analysis of systematic absences. All non-hydrogen atoms were refined anisotropically. No decomposition was observed during data collection. Details of the data collection and refinement are given in Table 1. Further details are provided in the Supplementary material.

Table 1
Crystallographic data and structure refinement parameters

	2	**3**
Formula	$C_{22}H_{14}Br_2N_2O_6Re_2S_3$	$C_{50}H_{38}N_6O_{11}Re_2S_6$
Molecular weight	1030.75	1463.62
Crystal system, Space group	monoclinic, $P2(1)/n$	triclinic, $P\bar{1}$
a (Å)	13.8647(17)	12.282(4)
b (Å)	12.7260(16)	14.072(4)
c (Å)	15.3535(19)	15.554(4)
α (°)		101.381(4)
β (°)	98.582(2)	104.464(4)
γ (°)		91.371(4)
V (Å^3)	2678.7(6)	2544.2(13)
Z	4	2
T (K)	89(2)	89(2)
λ (Å)	0.71073	0.71073
ρ_{calc} (Mg/m^3)	2.556	1.911
μ (mm^{-1})	12.288	5.068
F(000)	1904	1428
Crystal size (mm^3)	0.27 × 0.16 × 0.08	0.32 × 0.18 × 0.14
θ Range (°)	1.85–25.25	1.38–25.25
Index ranges	$-16 \leqslant h \leqslant 16$, $-15 \leqslant k \leqslant 15$, $-18 \leqslant l \leqslant 18$	$-14 \leqslant h \leqslant 14$, $-16 \leqslant k \leqslant 16$, $-18 \leqslant l \leqslant 18$
Number of reflections collected	39960	37764
Number of independent reflections (R_{int})	4861 (0.0433)	9222 (0.0251)
Data/restraints/parameters	4861/0/334	9222/0/676
Goodness-of-fit	1.100	1.023
R_1[a] [$I > 2\sigma(I)$]	0.0233	0.0207
wR_2[a] [$I > 2\sigma(I)$]	0.0499	0.0495
Largest difference in peak, hole (e Å^{-3})	0.997, −0.934	1.281, −0.629

[a] $R_1 = \sum||F_o| - |F_c||/\sum|F_o|$; $wR_2 = \{\sum[w(F_o^2 - F_c^2)^2]/\sum[w(F_o^2)^2]\}^{1/2}$.

3. Results and discussion

3.1. Synthesis and characterization of compounds **1–3**

Investigation of the interactions of 2,5-bis(benzylthio)-1,3,4-thiadiazole (**1**) with *fac*-$ReBr_3(CO)_3^{2-}$ yielded several unexpected results. Ligand **1** functioned in two coordination modes as a monodentate ligand solely through the nitrogen donor or as a bridging ligand through both nitrogen's on the thiadiazole ring. The general synthesis of compounds **1–3** prepared within are illustrated in Scheme 1.

Although the preparation of 2,5-bis(benzylthio)-1,3,4-thiadiazole (**1**) had been reported previously, the synthetic method reported in this work significantly improves both the yield and the purity of the compound [1]. The selection of methanol as the reaction solvent as opposed to previously reported dimethylformamide (DMF) was a better choice for reaction synthesis, isolation and purification. Compound **1** was prepared by reacting two equivalents of benzyl bromide and ~~1,3,4-thiadiazole-2,5-dithiol~~ in the presence of triethylamine in refluxing methanol and was isolated in near quantitative yields as an analytically pure colorless crystalline solid from the reaction solution as the reaction mixture slowly cooled from reflux to room temperature. Residual starting materials (benzyl bromide, ~~1,3,4-thiadiazole-2,5-dithiol~~, triethylamine) and the unwanted salt byproducts (triethylamine hydrogen bromide) from the reaction remained quite soluble in the methanol solution, which significantly improved and simplified the isolation and recrystalization of the product.

Compound **1** was reacted in equal molar equivalents directly with the common starting material $[NEt_4]_2$[*fac*-$ReBr_3(CO)_3$] (**2**, Scheme 1). It was anticipated that several possible species would be identified from the reaction mixture as **1** was expected to function as either a monodentate ligand from one of the three possible N or S donors or as a bidentate ligand through coordinating the sulfur in the thioether and a nitrogen of the thiadiazole ring with the $Re(CO)_3$ core. It was postulated that the isolated compounds could also contain two or three coordinated **1** ligands to the metal center. However, the formation of multiple coordinated **1** ligands with $[NEt_4]_2$[*fac*-$ReBr_3(CO)_3$] was not observed under the reaction conditions examined. The product *fac*-(di-μ-bromo)(μ-2,5-bis(benzylthio)-1,3,4-thiadiazole-κN:κ′N)bis(tricarbonyl rhenium (I)) (**2**) precipitated from the reaction mixture and was isolated as a colorless solid. In complex **2**, ligand **1** functions as a μ-**1**-κN:κN′ bridging two rhenium centers solely through the nitrogen's of the thiadiazole ring. Complex **2** has two additional coordinated μ bro-

mide ligands bridging the two rhenium centers to generate an overall neutral complex.

Initial experiments varying the concentration of **1** relative to the $[NEt_4]_2[fac\text{-}ReBr_3(CO)_3]$ starting material were conducted to investigate the possibility of inducing other species to form in solution. It was anticipated the reaction mixture may yield monomeric products such as $[fac\text{-}ReBr_2(CO)_3(\mathbf{1})]^-$, $fac\text{-}ReBr(CO)_3(\mathbf{1})_2$, and $[fac\text{-}Re(CO)_3(\mathbf{1})_3]^+$ or dimeric products $[fac\text{-}Re_2\mu\text{-}Br(CO)_3(\mu\text{-}\mathbf{1})_2]^+$ or $[fac\text{-}Re_2\mu\text{-}Br(CO)_3(\mu\text{-}\mathbf{1})_2]^{2+}$ in the course of the reaction. Treatment of $fac\text{-}[ReBr_3(CO)_3]^{2-}$ with excess **1**, which would most likely lead to monomeric species with multiple ligands per rhenium center or multiple bridging ligand between two metal centers did not yield such complexes in isolation. Treatment of $fac\text{-}[ReBr_3(CO)_3]^{2-}$ with limited concentration of **1**, which would most likely lead to bridging complexes with multiple rhenium centers per ligand bridging two, were not isolated as well. In all concentrations (0.5, 1, 2, 3, 5 equiv.) of **1** relative to the rhenium starting material, only compound **2** was isolated from the reaction mixture and no evidence for multiple products was found.

The ^1H and ^{13}C NMR of complex **2** were observed as expected for a symmetric 18 electron bimetallic complex. The coordinated ligand **1** spectral peaks were shifted slightly downfield of the uncoordinated ligand. The NMR spectra for **2** did not show any inequivalent protons or carbons differences based on the coordination geometry or orientation of **1**. The most significant shift in the ^{13}C spectra was the carbon in the thiadiazole ring which notably shifted from 164.8 in **1** to 174.3 ppm in complex **2**. The methylene proton and carbon signals of the benzylthioether were found to quite symmetrical in the ^1H (singlet, 4.37 ppm) and ^{13}C (single peak, 41.0 ppm) spectra. IR spectra of **2** showed the characteristic strong *fac* carbonyl stretches at 1937 and 1909.1 cm^{-1} shifted down from other $fac\text{-}Re(CO)_3$ stretches and equally strong stretches in the fingerprint region at 905 and 728.9 cm^{-1}. Complex **2** exhibited a strong broad UV absorbtion band at 272.0 nm hypsochromically shifted from free **1** at 294 nm. Determination of the complex parent ion with mass spectrometry of complex **2** was unsuccessful. A number of fragmentation peaks were observed, but could not utilized to clearly identify the M^+ parent ion.

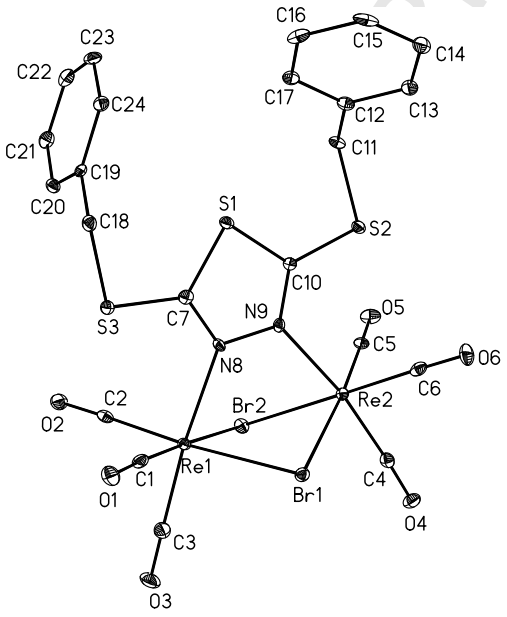

Fig. 1. View of the molecular structure for complex **2**. Thermal ellipsoids are shown with the 30% probability level. Non-functional hydrogen atoms were omitted for clarity.

The bridging nature of compound **1** through the nitrogen's of the thiadiazole ring to generate a bimetallic complex has been previous reported with some copper [3,11,12]. The copper complexes utilized a similar thioether analog compared to the benzyl analogs of ~~TDD~~ reported here. However, the copper complexes utilized a functional version with 2-methyl pyridine that provides two additional coordination donors to the metal centers. The bimetallic copper analogs also have additional μ bridging ligands in coordination complex in conjunction with the pyridyl thioether ~~TDD~~ ligand. Several variations of copper complexes were isolated that contained two μ bridging chloride or bromide ligands or a combination of μ bridging azide and bromide ligands. Unlike the copper complexes, complex **2** is a rare example of a bridging thiadiazole metal complex that does not utilize an additional function group to stabilize the rhenium metal centers. The unique formation of **2** may be due to the lack of available coordination sites as the substitutionally inert nature of the carbonyls in the $Re(CO)_3$ moiety.

In order to eliminate the possibility of bridging bromide ligands and generate a single non bridging rhenium complex, occupation of two of the available coordination sites of the labile bromide ligands in $fac\text{-}[Re(I)Br_3(CO)_3]^{2-}$ was achieved with a second bidentate ligand, picolinate. To compare the reactivity of **1**, a "2+1" style complex was prepared with **1** as a monodentate ligand. The

Table 2
Selected bond distances (Å) and bond angles (°) of **2** and **3**

2		**3**	
Br(1)–Re(1)	2.6379(6)	C(1)–Re(1)	1.916(3)
Br(1)–Re(2)	2.6477(6)	C(2)–Re(1)	1.926(3)
Br(2)–Re(2)	2.6172(6)	C(3)–Re(1)	1.914(3)
Br(2)–Re(1)	2.6294(6)	N(1)–Re(1)	2.175(2)
C(1)–Re(1)	1.900(5)	N(2)–Re(1)	2.203(2)
C(2)–Re(1)	1.916(5)	O(4)–Re(1)	2.137(2)
C(3)–Re(1)	1.929(5)		
C(4)–Re(2)	1.918(5)		
C(5)–Re(2)	1.915(5)		
C(6)–Re(2)	1.903(5)		
N(8)–Re(1)	2.205(4)		
N(9)–Re(2)	2.199(4)		
Re(1)–Br(1)–Re(2)	87.829(15)	C(3)–Re(1)–C(1)	87.05(13)
Re(2)–Br(2)–Re(1)	88.654(16)	C(3)–Re(1)–C(2)	87.39(13)
C(1)–Re(1)–C(2)	89.1(2)	C(1)–Re(1)–C(2)	86.33(13)
C(1)–Re(1)–C(3)	87.0(2)	C(3)–Re(1)–O(4)	94.82(10)
C(2)–Re(1)–C(3)	88.9(2)	C(1)–Re(1)–O(4)	174.93(10)
C(1)–Re(1)–N(8)	98.08(18)	C(2)–Re(1)–O(4)	98.45(11)
C(2)–Re(1)–N(8)	94.54(17)	C(3)–Re(1)–N(1)	91.38(11)
C(3)–Re(1)–N(8)	173.92(18)	C(1)–Re(1)–N(1)	99.74(11)
C(1)–Re(1)–Br(2)	177.82(14)	C(2)–Re(1)–N(1)	173.74(11)
C(2)–Re(1)–Br(2)	92.87(14)	O(4)–Re(1)–N(1)	75.53(8)
C(3)–Re(1)–Br(2)	92.03(16)	C(3)–Re(1)–N(2)	175.15(11)
N(8)–Re(1)–Br(2)	82.79(10)	C(1)–Re(1)–N(2)	95.43(11)
C(1)–Re(1)–Br(1)	94.58(14)	C(2)–Re(1)–N(2)	96.92(11)
C(2)–Re(1)–Br(1)	175.02(14)	O(4)–Re(1)–N(2)	82.37(8)
C(3)–Re(1)–Br(1)	94.68(16)	N(1)–Re(1)–N(2)	84.10(9)
N(8)–Re(1)–Br(1)	81.61(10)		
Br(2)–Re(1)–Br(1)	83.552(16)		
C(6)–Re(2)–C(5)	91.4(2)		
C(6)–Re(2)–C(4)	88.8(2)		
C(5)–Re(2)–C(4)	90.4(2)		
C(6)–Re(2)–N(9)	96.08(17)		
C(5)–Re(2)–N(9)	96.00(18)		
C(4)–Re(2)–N(9)	171.77(18)		
C(6)–Re(2)–Br(2)	177.77(15)		
C(5)–Re(2)–Br(2)	90.25(15)		
C(4)–Re(2)–Br(2)	92.68(15)		
N(9)–Re(2)–Br(2)	82.22(10)		
C(6)–Re(2)–Br(1)	94.79(15)		
C(5)–Re(2)–Br(1)	173.85(15)		
C(4)–Re(2)–Br(1)	89.62(14)		
N(9)–Re(2)–Br(1)	83.42(10)		
Br(2)–Re(2)–Br(1)	83.597(16)		

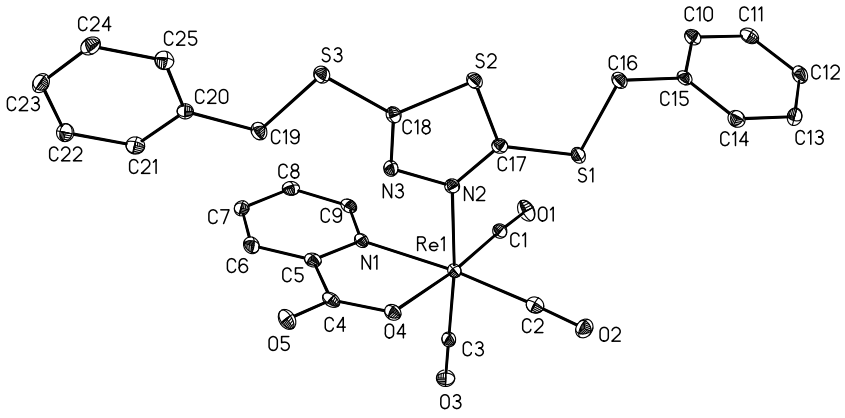

Fig. 2. View of the molecular structure for complex **3**. Thermal ellipsoids are shown with the 30% probability level. Non-functional hydrogen atoms and solvent molecule were omitted for clarity. Only one of the unique molecules in the asymmetric unit is shown.

product **3** was prepared in a two step one pot process. The intermediate *fac*-Re(I)$(OH_2)(CO)_3$(picolinate) (**4**) was prepared similarly to previously reported procedures [28]. The formation and completion of the intermediate product **4** (18.7 min) was characterized by HPLC after the reaction was heated for 1 h prior to the addition of **1**. Introduction of **1** (24.4 min) to the reaction mixture containing **4** resulted in the formation of a new species, [*fac*-Re$(CO)_3$(picolinate)(5-bis(benzylthio)-1,3,4-thiadiazole)] (**3**; 23.5 min). The product was isolated and from the reaction mixture and characterized by standard analyses.

The ^{1}H NMR of **3** illustrated unexpected results in interactions of **1** in complex **3**. The most significant observation in the spectra are the methylene carbons of the benzylthioether group in **1**. The methylene carbons had two unique signals depending on which side of the coordinated **1** they were located. As expected, the methylene groups would be inequivalent due to asymmetrical coordination nature of the thiadiazole ring. The first set of methylene protons were identified as a singlet at 4.33 ppm, while the second methylene group exhibited inequivalent H_a and H_b, as a doublet–doublet pattern at 3.98 and 4.18 ppm (J = 12.9). The steric orientation of coordinated **1** in complex **3** is believe to contort the methylene protons of the thiobenzyl analogs to interact with the equatorial plane of the picolinate ligand and CO ligands affecting the magnetic environment of each proton. The ^{13}C NMR further illustrated the asymmetrical coordination of **1** in complex **3**. Two sets of signals were assigned to the methylene carbons at 41.2 and 38.4 ppm. The asymmetry of **1** in **3** was evident in a slight distinction in the aromatic carbons as they also showed a modest shift in the two different benzyl portions of **1**. Complex **3** had a slight hypsochromic shift in the UV/Vis absorbance spectra from 286.9 nm compared to the free ligand at 294 nm. The shift in absorption peak in **3** was not as nearly pronounced as in the bridging complex **2**. Complex **3** illustrated a similar IR spectra to compound **2**, where the *fac*-Re$(CO)_3$ *exhibited strong stretches at 2017 and 1889. Additional stretches identified were attributed to the picolinate and* **1** at 1637, 1598, 1349, 774, and 691. Mass spectrometry of **3** clearly identified the M^+ ion at 745.9 in acidic condition. A fragmentation peak at 331 was observed and attributed to the free **1**, which probably dissociated upon ionization of the compound.

3.2. X-ray characterization

Single crystals of **2** were isolated by slow diffusion of pentane into a dichloromethane solution. Characterization by single crystal X-ray diffraction yields the complex shown in Fig. 1. This complex crystallizes in the monoclinic $P2(1)/n$ space group and the structure shown is symmetry unique with all atoms lying on general positions (Table 1). The major bond distance and angles in compound **2** are reported in Table 2. The Re–Re distance (3.66 Å) is too long to be considered a bond. The thiadiazole bridges the Re–Re vector with N–Re distances of ca. 2.2 Å. The strain of this chelation affects the bridging bromide atoms which are canted out of the Re–Re plane with Re–Br–Re angles of 87.8° and 88.6° and a Br–Br distance of ca. 3.509 Å. This is seen in other $Re_2(CO)_6$ chalcogenide bridged complexes where the strain of the chelation also distorts the geometry away from the simple orthogonal system [29–33]. The packing structure of **2** also has a close arene–thiadiazole interaction (3.56 Å from S1 to centroid of the C18–C24 ring) which is similar to slipped π–π interactions.

The complex **3** was isolated in crystalline form from a mixed acetonitrile water solution. The complex is less symmetric and was solved in the $P\bar{1}$ space group with two independent complex molecules and a H_2O molecule in the asymmetric unit (Table 1). The structure, shown in Fig. 2, displays monodentate ligation by the thiadiazole and the other octahedral sites are occupied by the picolinate ligand. Similar bond lengths (Table 2) are seen in **2** and **3** as well as in related complexes, e.g. ~~(picolinate-COOH)~~Re$(CO)_3$(imidazole) [34]. In **3**, however, the methylene groups are rotated to elongate the thiadiazole parallel to the picolinate–Re–CO plane. This enables closer packing of the phenyl and thiadiazoles with arene–thiadiazole π–π interactions of ca. 3.8–3.9 Å (centroid to centroid). This distance is long for a π–π interaction, thereby indicating that this interaction is very weak. The water molecule links two complexes together via strong hydrogen bonding with the picolinate ligand.

4. Conclusions

2,5-Bis(benzylthio)-1,3,4-thiadiazole (**1**) was found to generate two types of complexes when reacted with *fac*-$[NEt_4]_2$[Re$(CO)_3Br_3$]. A single ligand of **1** was found to bridge two rhenium centers to yield **2** even in the presence of excess **1**. A monodentate complex **3** with **1** was prepared using a "2+1" approach with the bidentate picolinate as a coligand. In both complexes **2** and **3**, ligand **1** coordinated through the nitrogen in the 1,3,4-thiadiazole ring only. No evidence was suggested that the ligand would coordinate through either of the thioether donors in **1**. Compound **1** and analogs with rhenium may provide interesting chemistry in potential radiopharmaceutical, bioinorganic and material applications.

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Appendix A. Supplementary material

CCDC 682999 and 683000 contain the supplementary crystallographic data for **2** and **3**. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ica.2008.06.018.

References

[1] X. Shen, Z. Li, H.Y. Zhang, H.B. Qiao, Q.A. Wu, H.Y. Wang, Y. Zu, J. Phys. Chem. Solids 66 (2005) 1755.
[2] M. Al-Talib, H. Tashtoush, Indian J. Chem., Sect. B 38B (1999) 1374.
[3] S. Tandon, L. Thompson, J. Bridson, J. Chem. Soc., Chem. Commun. 9 (1993) 804.
[4] A. Fabretti, G. Franchini, G. Peyronel, Transit. Met. Chem. 7 (1982) 105.
[5] Z. Li, S. Du, X. Wu, Polyhedron 24 (2005) 2988.
[6] V. Ng, S. Kuan, Z. Weng, W. Leong, J. Vittal, L. Koh, G. Tan, L. Goh, J. Organomet. Chem. 690 (2005) 2323.
[7] X. Zhang, H. Chen, C. Ma, C. Chen, Q. Liu, Dalton Trans. 33 (2006) 4047.
[8] C. Ma, J. Zhang, F. Li, R. Zhang, Eur. J. Inorg. Chem. 13 (2004) 2775.
[9] C. Ma, F. Li, Q. Jiang, R. Zhang, J. Organomet. Chem. 689 (2004) 96.
[10] M. Castano, A. Sanchez, J. Casas, J. Sordo, E. Castellano, E. Eduardo, Inorg. Chim. Acta 201 (1992) 83.
[11] S. Tandon, L. Thompson, M. Manuel, J. Bridson, Inorg. Chem. 33 (1994) 5555.
[12] S. Tandon, L. Chen, L. Thompson, J. Bridson, Inorg. Chem. 33 (1994) 490.
[13] S. Gopinathan, S. Deshpande, C. Gopinathan, Transit. Met. Chem. 18 (1993) 585.
[14] S. Gopinathan, C. Gopinathan, S. Pardhy, S. Tavale, V. Puranik, S. Krishnan, Inorg. Chim. Acta 195 (1992) 211.
[15] H. Tannai, K. Tsuge, Y. Sasaki, Inorg. Chem. 44 (2005) 5206.
[16] A. Mamo, A. Pappalardo, M. Clasadonte, Molecules 8 (2003) 310.
[17] Q. Ye, W. Ye, S. Zhang, Huaxue Yanjiu 16 (2005) 17.
[18] E. Marchesi, A. Marchi, L. Marvelli, M. Peruzzini, M. Brugnati, V. Bertolasi, Inorg. Chim. Acta (2005) 352.
[19] B. Tzeng, Y. Wu, G. Lee, S. Peng, New J. Chem. 31 (2007) 199.
[20] T. Mindt, H. Struthers, L. Brans, T. Anguelov, C. Schweinsberg, V. Maes, D. Tourwe, R. Schibli, J. Am. Chem . Soc. 128 (2006) 15096.
[21] D. van Staveren, P. Benny, R. Waibel, P. Kurz, J. Pak, R. Alberto, Helv. Chim. Acta 88 (2005) 447.
[22] R. Alberto, R. Schibli, P. Schubiger, U. Abram, T. Kaden, Polyhedron 15 (1996) 1079.
[23] H. Hope, Prog. Inorg. Chem. 41 (1994) 1.
[24] SMART: v.5.630, Bruker Molecular Analysis Research Tool, Bruker AXS, Madison, WI, 2001.
[25] SAINTPLUS: v. 6.45a and v.7.23a, Data Reduction and Correction Program, Bruker AXS, Madison, WI, 2003/2004.
[26] SADABS: v.2.01, v. 2004/1 An Empirical Absorption Correction Program, Bruker AXS Inc., Madison, WI, 2004.
[27] G.M. Sheldrick, SHELXTL: v. 6.14, Structure Determination Software Suite, Bruker AXS Inc., Madison, WI, 2004.
[28] R. Schibli, R. Labella, R. Alberto, E. Gracia-Garayoa, K. Ortner, U. Abram, P. Schubiger, Bioconjugate Chem. (2000) 345.
[29] I. Bernal, J.L. Atwood, F. Calderazzo, D. Vitali, Isr. J. Chem. 15 (1977) 153–159.
[30] F. Calderazzo, D. Vitali, R. Poli, J.L. Atwood, R.D. Rogers, J.M. Cummings, I. Bernal, J. Chem. Soc., Dalton Trans. (1981) 1004–1009.
[31] J. Korp, I. Bernal, J.L. Atwood, F. Calderazzo, D. Vitali, J. Chem. Soc., Dalton Trans. (1979) 1492–1496.
[32] I. Bernal, J.L. Atwood, F. Calderazzo, D. Vitali, Gazz. Chim. Ital. 106 (1976) 971–991.
[33] W. Baratta, I. Bernal, F. Calderazzo, J.D. Korp, L.S. Magill, F. Marchetti, D. Vitali, Gazz. Chim. Ital. 126 (1996) 469–474.
[34] S. Mundwiler, M. Kundig, K. Ortner, R. Alberto, Dalton Trans. (2004) 1320–1328.